Mehdi Razzaghi-Abyaneh
Mahendra Rai *Editors*

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Preface

Plants are rich sources of beneficial secondary metabolites which are attractive as flavors, fragrances, pesticides, pharmaceuticals, and antimicrobials. The use of plants for combating different fungal pathogens of humans and animals dates back to the beginning of human civilization. Plants and plant-derived products are well known in 'Ayurveda' (ancient science of life) and in other traditional systems as antifungal remedies. They are important sources for a diverse range of antifungal metabolites. The extracts and oils from plants have usually no side effects and as a unique advantage, they are within the reach of the common people all over the world. Aside from a brilliant role to combating fungal diseases of human beings, plant-derived natural products can also be used for the management of phytopathogens of fungal origin. It is a natural way of coping with fungal infections.

Worldwide occurrence of fungal infections, especially from commensal pathogens such as *Candida*, has been dramatically increased in recent years due to a continuous increase in immunosuppressive conditions like AIDS, organ transplantation, cancer, and diabetes mellitus. Increasing trends of health organizations and pharmaceutical industries to use plants as safe and effective alternative sources of synthetic antifungals is due to major problems of slow growing and high costs of synthetic pharmaceutics, their life-threatening side effects, rapid increasing of new fungal infections, and the dramatic emergence of multidrug resistance fungal pathogens. World trade in medicinal plants is now more than 43 billion dollars and is predicted to reach to 5 trillion dollars in 2050.

The main goal of this book is to provide information to readers regarding use of different medicinal plants and their bioactive metabolites in combating various fungal diseases of humans, animals, and plants.

The book has been divided into four parts: Part I incorporates global distribution of antifungal compounds, Part II deals with antifungal activities of plants and plant-derived natural products, Part III includes plants used in 'Ayurveda' and traditional systems for treatment of fungal diseases, and Part IV discusses the use of plant-derived products to protect fungal diseases of plants/fruits.

The book will be of utmost importance to students, researchers, and teachers of medicine, botany, mycology, microbiology, and pharmacology. The readers should find the book full of information and reader-friendly.

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Part I
Global Distribution of Antifungal Compounds

Chapter 1
Antifungal Compounds from Latin American Plants

Laura Svetaz, Marcos Derita, Ma. Victoria Rodríguez, Agustina Postigo, Estefanía Butassi, Ma Victoria Castelli, Maximiliano Sortino, Elisa Petenatti and Susana Zacchino

Abstract Latin American region comprises six of the most biologically diverse countries in the world, thus constituting one of the planet areas richest in biodiversity. Some efforts have been made to screen plants of the whole region and also of each country, but the amount of studies in each country is not correlated with its vegetal diversity. Regarding antifungal compounds isolated from this region, many structural types that have demonstrated antifungal properties are presented here. These previous studies are important starting points for the development of new antifungal drugs. However, most studies are preliminary and begin and end with in vitro assays without comparative toxicity studies or in vivo tests. Few of them deepen the mechanisms of action and with rare exceptions, no clinical studies were carried out. A close collaboration among Latin American countries one each other and with the whole world is highly needed and might help in the discovery of new natural antifungal structures from Latin American plants.

1.1 Introduction

Fungi have emerged over the past two decades as major causes of human infections, especially among immunocompromised hosts. They produce serious invasive mycoses in individuals submitted to organ transplants or antineoplastic

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chemotherapy, those with acquired immunodeficiency syndrome (AIDS), extremely aged persons and patients in intensive care units, among others (Espinel-Ingroff 2009; Mathew and Nath 2009), and have an enormous impact on morbidity and mortality. They also produce superficial fungal infections (those involving the skin and mucosal surfaces) not only in immunocompromised hosts but also in healthy individuals, including children of less developed nations that receive deficient sanitary attention and education, highly diminishing the quality of their lives (Weitzman and Summerbell 1995). Although it appears to be a big armamentarium of antifungal drugs in clinical use, in fact only a modest number of drugs, derived from six antifungal classes, are available (Mathew and Nath 2009) and most of them have been isolated from natural sources (mainly fungi) or are natural-derived compounds. So, the polyenes, nystatin and amphotericin B, were isolated from *Streptomyces* spp. (Gold et al. 1955); the tricyclic spirodiketone griseofulvin (Brian et al. 1949) was isolated from *Penicillium griseofulvum*, and among the more recently approved antifungal drugs, caspofungin acetate (Merck & Co. Inc.) was obtained by semi-synthesis of pneumocandin B, which, in turn, was isolated from the aquatic fungus *Glarea lozoyensis* (Peláez et al. 2000); micafungin sodium and anidulafungin were derived from the lipopeptide FR901379, which was isolated from the fungus *Coleophoma empetri*, a plant pathogen associated with post-harvest fruit rot in cranberries (Espinel-Ingroff 2009).

The limited availability of effective antifungal agents, the increasing resistance of fungi to the existing drugs and the very few new drugs in development have led to a general consensus that new antifungal structures are highly needed (Mathew and Nath 2009).

Among the different possible sources of new antifungal drugs, natural products maintain a great interest because they provide unlimited opportunities for the isolation of new antifungal compounds due to their unmatched availability of chemical diversity (Odds 2005; Cos et al. 2006).

An analysis of the papers published in *Journal of Natural Products* in the last decade reveals that among the different types of organisms investigated for antifungal properties: 41 % were plants; 34 %, fungi; 20 %, marine organisms and 5 %, bacteria

This analysis evidences the interest of researchers in natural sources for the discovery of antifungal compounds in recent years, that is in contrast to the trend showed by most big pharmas which abandoned antifungal drug discovery programmes based on natural products, including plant biodiversity (Rouhi 2003). In addition, it is clear that among natural resources, plants have been the most used source for antifungal research.

Regarding the evolution of papers devoted to the search of antifungal compounds in plants, the analysis of two Journals devoted to natural products (*Journal of Ethnopharmacology* and *Planta Medica)*, showed that 221 papers on antifungal screening of plants were published in the JEP in the last three decades, increasing from 16 in 1981–1990, 66 in 1991–2000 up to 139 in 2001–2010. Regarding *Planta Medica,* 151 papers on antifungal plants were published in the same period,

24 in the first 10 years (1981–1990), 43 in the second (1991–2000) and 84 in the third (2001–2010) (source: unpublished personal analysis by the authors).

The first important concern within a programme of discovery of new plants with antifungal properties is the selection of species to be submitted to biological evaluation.

According to a recent review of Ríos and Recio (2005), a wide range of criteria were followed to select plants to be submitted to antimicrobial studies. Some researchers investigated plants growing in a specific region or country; others focused on plant families; others on ethnopharmacology and many others on the *random* screening of plants.

Particularly, the study of antifungal plants delimited to a certain region or country is clearly correlated with the region biodiversity, accessibility, research possibilities and many others.

Among the flora of different regions of the world, Latin America represents one of the wealthiest sources of material with pharmacological activity due to its biodiversity (Brandão et al. 2008). It possesses a very high number of vascular plants existing a recent evidence that neotropical forests located in Latin America possess the highest diversity of plants in the world (Berry 2002). In addition, some factors critically distinguish the medicinal plants of Latin America. (1) This region possesses a huge unexplored biodiversity (Cruz et al. 2007); (2) there is a rich tradition of use of medicinal plants (Gupta 1995, 2008); and (3) the ethnopharmacological knowledge has been tightly kept or transmitted by many indigenous populations still living in this region (Correia 1984; Cleaves 2001; Portillo et al. 2001; Coelho de Souza et al. 2004; Scarpa 2004; Goleniowski et al. 2006; Cruz et al. 2007; Estévez et al. 2007)

This chapter aims to examine the antifungal plants detected within Latin America region. The information has been organized into sections that include a general information about the vegetal diversity in Latin America, the antifungal plants of each country referring first to the screening of crude extracts and then to antifungal isolated constituents.

1.2 General Information About Latin America Region and its Vegetal Diversity

1.2.1 Definition of the Term Latin America

Latin America refers to regions of the Americas extending south of the US, where Romance languages are spoken, specifically Spanish, French and Portuguese (Ardao 1993).

Table 1.1 Total number of plant species that inhabit the different Latin American countries including the endemisms when known

Country	No. of spp.	No. of endemisms
Brasil	40,982	8,000
Colombia	40,000	
México	26,000	10,000
Venezuela	30,000	
Ecuador	18,137	5,400
Bolivia	17,367	
Perú	13,300	5,354
Costa Rica	12,119	600
Cuba	11,000	6,300
Panamá	10,444	1,200
Argentina	9,690	1,906
Guatemala	9,317	686
Paraguay	8,000	
Nicaragua	5,796	79
Honduras	7,525	
Rep. Dominicana and Haití	5,400	1,800
Chile	5,000	151
Uruguay	2,500	

1.2.2 Latin American Countries Vegetal Diversity

Latin American region comprises six of the most biologically diverse countries in the world (Brazil, Colombia, Ecuador, Mexico, Venezuela and Peru), thus constituting one of the areas of the planet richest in biodiversity (Bovarnick et al. 2010). Only the Amazon region contains approximately 16 % of all plant species that exist today on the Earth (that is calculated in 300,000–500,000) (Hammond 1995), and this wealth is higher towards the west of the region (Sanz-Biset et al. 2009). Table 1.1 summarizes the number of total species of each Latin American country and the number of endemisms (number of species that are unique to a region or country) which, when known, is a highly important datum.

1.2.3 Value of the Ethnomedical Information for the Discovery of Plants with Antifungal Properties in Latin American Countries

Within a multidisciplinary collaborative project within the Organization of the American States (OAS) carried out during the period 2001–2004, plant extracts from seven countries (Argentina, Bolivia, Brazil, Colombia, Costa Rica,

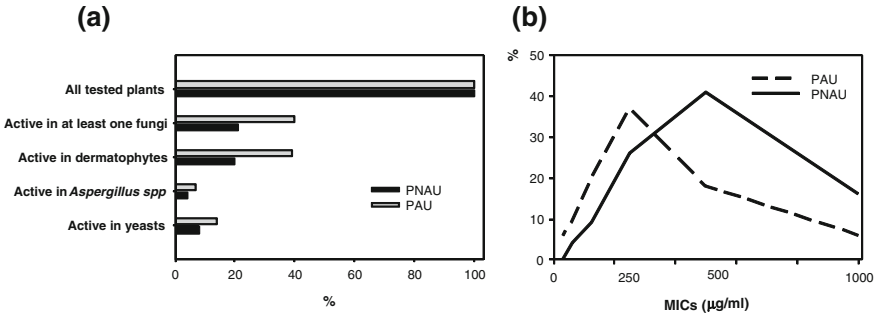


Fig. 1.1 **a** Percentage of active extracts against: at least one fungal species; dermatophytes; *Aspergillus* spp: and yeasts, within PAU (plants with ethnopharmacological uses related to mycoses) groups and PNAU (plants without ethnopharmacological uses related to mycoses). **b** Percentage of all MICs $\leq$ 1,000 µg/mL (considered 100 %) acting against dermatophytes that fall into each of the different MIC values (in µg/mL): from 1,000 to 15.62, within PAU or PNAU group of plants

Guatemala and Panama) were screened for antifungal properties with a standardized methodology against the same fungi, in a single laboratory.

Results showed (Svetaz et al. 2010) that a significant higher chance of detecting plants with antifungal activity against at least one fungus was found within the group named *p*lants with related reports of *a*ntifungal ethnopharmacological *u*ses (PAU) group (40 %) than within the *random* group named *p*lants with *n*ot related reports of *a*ntifungal ethnopharmacological *u*ses (PNAU, 20 %, $p < 0.01$) (Fig. 1.1a). Within the detected antifungal plants from both groups, plants of the PAU group tend to display better activities (lower MICs) only against dermatophytes than those of PNAU group ($p < 0.05$) (Fig. 1.1b), demonstrating that the ethnopharmacological approach is useful in guiding the discovery of antifungal Latin American plants mainly for infections in which the pathological expression is obvious (superficial infections due to dermatophytes).

1.2.4 Random Screening of Latin American Plants for Antifungal Activities

Some papers devoted to the screening of random selected antifungal Latin America plants were published (Gutkind et al. 1981; Freixa et al. 1998). A recent paper reported the screening of 151 Latin American plants against *Sporothrix schenckii* and *Fonsecaea pedrosoi* which are the cause of subcutaneous chronic mycoses occurring in tropical and subtropical regions. Twenty-eight plants showed activity (Gaitan et al. 2011), opening an avenue for the further isolation of new antifungal compounds to treat these mycoses difficult to eradicate.

1.3 Latin American Countries' Plants as Sources of Antifungal Metabolites

1.3.1 Argentina

Although the vegetal diversity of Argentina is moderate (~10,000 *spp*), it exists a high endemism (20 %). There are some studies devoted to *random* screening (Quiroga et al. 2001; Lima et al. 2011) and others following the ethnopharmacological approach (Zacchino et al. 1998; Muschietti et al. 2005; Petenatti et al. 2008).

Below, some selected antifungal compounds isolated from Argentinean plants are detailed grouped by vegetal families.

1.3.1.1 Asteraceae

From *Heterothalamus alienus* (Spreng) Kuntze (common name "romerillo") collected in the central Cordoba province (Pacciaroni et al. 2008), sakuranetin **1,** (2*R*, 3*R*)-dihydroquercetin-7,4′-dimethyl ether **2** and 3-acetoxy-5.7.4′-trihydroxyflavanone **3**, with moderate antifungal properties against dermatophytes, were isolated. From *Baccharis darwinii* of the southern Chubut province, the prenylated coumarin 5′-oxoaurapten **4** was highly active only against dermatophytes (Kurdelas et al. 2010). From *Tagetes mendocina,* the thiophenes 5-(4-hydroxy-1-butynyl)-2, 2′-bithienyl (BBTOH) (**5**) and its acetate (BBTOAc) (**6**) showed high activity against dermatophytes (Lima et al. 2009).

1.3.1.2 Fabaceae

From *Geoffroea decorticans* (Gill.et Arn.) Burk., collected in arid regions of Tucuman province, two 5′-prenylisoflavanones **7** and **8** were isolated when tested against four species of *Aspergillus* genus (Quiroga et al. 2008). From *Zuccagnia punctata* Cav., a monotypical sp., endemic to central and western semi-arid regions of Argentina, 2′,4′-dihydroxy chalcone **9** and 2′,4′-dihydroxy-3′-methoxy chalcone **10** were isolated. Studies on mechanism of action showed that **9** acted by a different mode of action that the antifungal drugs in current use. Its essential oil (EO) also showed antifungal activity, and although (-)-5,6-dehydrocamphor was the main component, the activity would be probably due to carvacrol, thymol and linalool (Alvarez et al. 2012). From *Dalea elegans,* which is the only species growing in Cordoba province from the exclusive American genus *Dalea*, pinocembrin **11** showed to inhibit rhodamine 6G efflux in both azole-sensitive and azole-resistant *Candida albicans*, effect that was enhanced in the presence of fluconazole (Peralta et al. 2012).

1.3.1.3 Gentianatae

Xhantones **12–15** from *Gentianella multicaulis* (Gillies ex Griseb.) Fabris (common name "nencia"), collected at 2,750 m over sea level at San Juan province, showed moderate activity against dermatophytes (Lima et al. 2012).

1.3.1.4 Phytolaccaceae

From *Phytolacca tetramera* Hauman (common name "ombusillo"), an endemic species in critic risk (Hernández et al. 1998) of Buenos Aires province, phytolaccoside B **16** was isolated showing high antifungal activities (Escalante et al. 2002). It produced hyphal malformations similar to those produced the inhibitors of the synthesis of (1,3)β-D-glucan, major polymer of the fungal cell wall. However, it did not inhibit the synthesis of this glucan, but produced a notable enhancement of the fungal chitin content increasing the thickness of its cell wall and therefore leading to fungal death (Escalante et al. 2008).

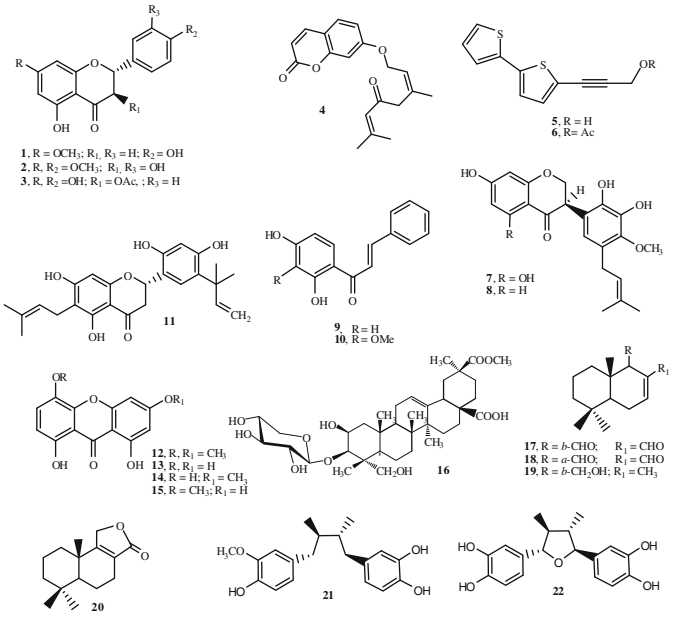
1.3.1.5 Polygonaceae

Several species of *Polygonum* genus belonging to Persicaria section were evaluated for antifungal properties. From *Polygonum ferrugineum*, 2′,4′-dihydroxy-6′-methoxychalcone (cardamonin, not shown) displayed the highest antifungal activity and selectivity towards *Epidermophyton floccosum*. In addition, it induced *Neurospora crassa* malformations suggesting that it could act by inhibiting the fungal cell wall (López et al. 2011). From the most active extract of *Polygonum acuminatum* Kunth., polygodial **17**, isopolygodial **18**, drimenol **19** and confertifolin **20** were isolated. Leaves of Autumn possess the highest content of **17** and the lowest MICs (Derita et al. 2008, 2009). In turn, the antifungal behaviour of *Polygonum persicaria* L. could be attributed to drimane **17** too, but also to cardamonin (Derita and Zacchino 2011). The drimane-type dialdehyde **17** was first isolated from *Polygonum hydropiper* L. in Australia (Barnes and Loder 1962), and its activity against *C. albicans* was previously reported (Mc Callion et al. 1982; Lee et al. 1999). Studies on mode of action of **17** showed that it uncouples mitochondrial ATP synthesis by affecting the electrical properties of the membrane surface and consequently collapsing the membrane potential (Castelli et al. 2005) which constitutes a biochemical basis for explaining its antifungal activity.

1.3.1.6 Zygophyllaceae

From *Larrea nitida* Cav. collected at San Juan province, 3′-methyl nor-dihydroguaiaretic acid **21** and *meso* 7*S*, (8*S*, 7′*R*, 8′*R*)-3,4,3′,4′-tetrahydroxy-7,7′-epoxylignan **22** showed strong activity against standardized strains of

dermatophytes and clinical strains of *Candida* spp. and *Cryptococcus neoformans* (Agüero et al. 2011).



1.3.2 Brazil

Brazil is considered to have the greatest biodiversity of any country on the planet, and the 20 % of the Angiosperms of the whole world are found in Brazil. There are some studies devoted to either antifungal *random* screening (Fenner et al. 2005; Teixeira et al. 2005; Silva Jr et al. 2008) or following the ethnopharmacological approach (Cruz et al. 2007; Braga et al. 2007).

Below, some selected antifungal compounds from Brazilian plants are detailed grouped by vegetal families.

1.3.2.1 Annonaceae

From *Porcelia macrocarpa* (Warm.) R.E.Fries, the alkaloids cleistopholine **23** and its methoxy derivative **24** showed high antifungal activity against *Cladosporium* spp. (Lago et al. 2007).

1.3.2.2 Asteraceae

From *Acmela brasiliensis* Spreng (synonym *Wedelia paludosa*, common name margaridão) collected at Santa Catarina State, kaurenoic acid **25** showed low activity only against *E. floccosum* (Sartori et al. 2003).

From the aerial parts of *Pterocaulon polystachyum* DC collected at Rio Grande do Sul State, a mixture of 6-hydroxy-7-(3′-methylbutyl-2′-en-oxy)-coumarin (prenyletin) **26** and prenyletin methyl ether **27** showed moderate activity only against dermatophytes (Stein et al. 2006).

In turn, 2-hydroxy-4,6-dimethoxyacetophenone (xanthoxyline) **28** was isolated from *Phyllanthus sellowianus* Müll. Arg. (common name sarandí blanco) and *Sebastiania schottiana* Müll. Arg. (common name sarandí negro), from Rio Grande do Sul and Santa Catarina States, respectively, showing to possess fungicide (rather fungistatic) activity against *Microsporum canis* (Lima et al. 1995).

1.3.2.3 Lythraceae

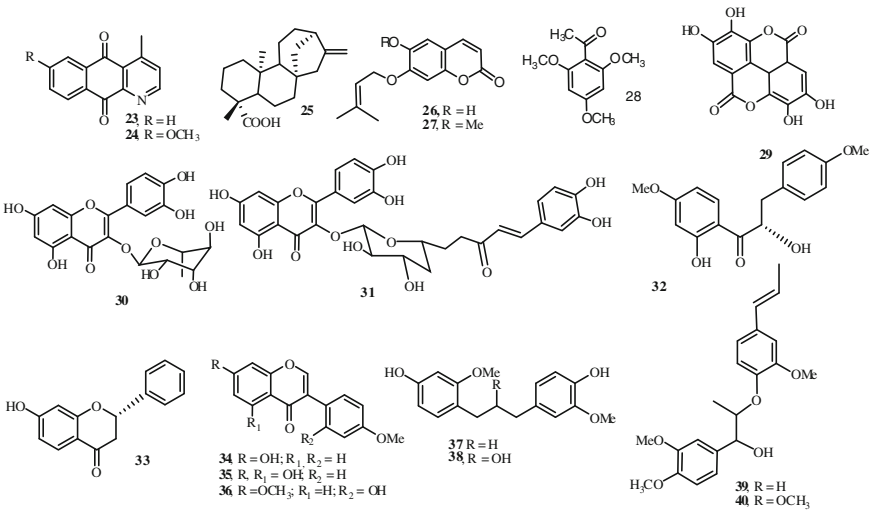
From *Lafoensia pacari* St.-Hill. of Mato Grosso State, the antifungal ellagic acid **29** was isolated (Silva Jr. et al. 2010). Interesting enough, sorbitol (Frost et al. 1995) and *Neurospora crassa* assays (Fukuda et al. 1991) suggested that it could act through the inhibition of the fungal cell wall synthesis or assembly. This mechanism represents an ideal mode of action for antifungal drugs since human cells are devoid of walls.

1.3.2.4 Melastomataceae

From *Tibouchina grandifolia* (common name orelha de onça) collected at Espirito Santo State, quercitrin **30** and quercetin-3-O-*β*-D-(6″-*E*-*p*-cumaroyl)-glucopyranoside **31** showed activity against *Cladosporium cucumerinum* (Kuster et al. 2009).

1.3.2.5 Myristicaceae

From *Virola surinamensis* (from Amazonas), dihydrochalcone **32**, flavanone **33**, isoflavonoids **34**–**36** and 1,3-diarylpropanes **37**, **38** showed the best antifungal activities against *Cladosporium cladosporioides* (Lopes et al. 1999). In turn, 7,8-*threo* alcohols of 8.O.4′-neolignan structure, **39**, **40** from *V. surinamensis* showed moderate activity against *E. floccosum* but not against yeasts or *Aspergillus* spp. The *erythro* analogues found in *Myristica fragrans* showed better activity (Zacchino et al. 1997).



1.3.2.6 Piperaceae

Four chromenes (**41**–**44**) and a prenylated benzoic acid **45** isolated from *Piper aduncum* were active against mutants of *Saccharomyces cerevisiae* lacking DNA cleavage repair mechanism (Baldoqui et al. 1999). Although the activity was weak, they were selective towards this mutant being inactive against the wild-type strain, laying the groundwork to look for new compounds with antifungal activities in the Piperaceae family. Amides **46** and **47** from *Piper hispidum* and *Piper tuberculatum* demonstrated high antifungal activity in bioautographic methods against *Cladosporium sphaerospermum*, while analogues including those with *cis* geometry in their side chain showed 5 or 10 times lower activity (Debonsi et al. 2000).

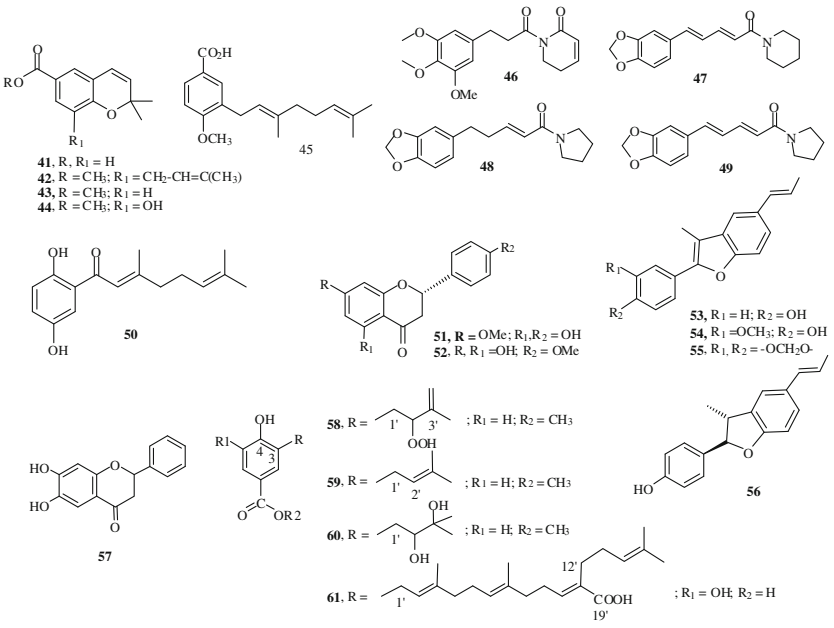
Bioassay-guided fractionation of *Piper arboreum* using *C. sphaerospermum* and *C. cladosporioides* led to the isolation of amides **48** and **49** which showed the best antifungal activity among the 12 other isolated amides. Unfortunately MIC values were not reported (Vasques da Silva et al. 2002).

In a further paper, the same authors isolated the prenylated hydroquinone **50** and the flavanone sakuranetin **51** from *Piper crassinervium* against *C. sphaerospermum* and the same compounds *plus* naringenin **52** when used *C. cladosporioides* (Danelutte et al. 2003).

The bioassay-guided fractionation of the EtOH–H_2O extract of the leaves of *Piper regnelli* (common name pariparoba) from Maringá, Parana State, Brazil, against four *Candida* spp. led to the isolation of four compounds, the eupomatenoids **53**–**55** and the lignin (+)-conocarpan **56** which showed high fungistatic as well as fungicide activities against *C. albicans*, *Candida krusei* and *Candida tropicalis* with MICs below 20 μg/mL (Pessini et al. 2005). In a further paper,

Koroishi et al. reported that **54** was active also against *Trichophyton rubrum* with MIC 6.2 μg/mL (Koroishi et al. 2008).

From leaves of *P. aduncum* and *Piper hostmannianum* C. DC., the flavanone pinocembrin **57** and three prenylated benzoic acid derivatives **58**–**60** showed antifungal activities against *C. sphaerospermum* and *C. cladosporioides* (Lago et al. 2009). Instead, other prenylated benzoic acid derivatives such as caldensinic acid **61** did not show antifungal activity against the same fungi (Freitas et al. 2009).



1.3.2.7 Polygonaceae

From *Polygonum punctatum* Ell. (common name "herva do bicho"), from the state of Minas Gerais (de Almeida Alves et al. 2001), the sesquiterpene polygodial **17** was isolated against *C. sphaerospermum.* As it was stated above, there are other reports on the antifungal activity of native antifungal Polygonum plants from Argentina containing **17** and also studies on its mechanism of action. From *Polygonum spectabile,* collected at Minas Gerais State, 2′,4′-diOH-3′,6′-dimethoxy chalcone (not shown) was found to be antifungal only against dermatophytes (Brandão et al. 2010).

1.3.2.8 Sapindaceae

From *Serjania salzmanniana* Schlecht., three oleanolic monodemosidic saponins **62–64** showed high activity mainly against *C. neoformans* (Ekabo et al. 1996).

1.3.2.9 Winteraceae

From *Drimys brasiliensis* collected at Santa Catarina State, other drimanes related to **17**, 1-*β*-(*p*-methoxycynammoyl)-polygodial **65** and 1-*β*-(*p*-cumaroyloxy)-polygodial **66** showed moderate antifungal activity only against dermatophytes (Malheiros et al. 2005).

1.3.3 Chile

Chile is divided into fifteen regions and has a rich diversity in spite of its small surface. There are some studies devoted to *random* antifungal screening (Avello et al. 2012) or following the ethnopharmacological approach (Morales et al. 2008). Below, some antifungal compounds isolated from Chilean plants.

1.3.3.1 Phytolaccaceae

The activity of the saponin-rich extract of *Phytolacca dioica* L. berries, collected in the surroundings of Santiago, was low, but its acid hydrolysate and its major aglycone, phytolaccagenin (not shown), showed promising antifungal potency against ATCC strains of *C. albicans* and *C. neoformans*, and against clinical isolates of these fungi (Di Liberto et al. 2010).

1.3.4 Colombia

Most research of antifungal compounds for human beings at Colombia is related to the analysis of antifungal activities of essential oils grouped by families such as Verbenaceae (*Lippia* genus) (Mesa-Arango et al. 2008, 2009; Betancur-Galvis et al. 2011), Piperaceae (Mesa-Arango et al. 2007), Asteraceae (Zapata et al. 2010), Lamiaceae and others.

1.3.5 Costa Rica

This country is not big, but over 10,000 species of vascular plants (the main category of plant species) have been recorded in Costa Rica. In addition, its tropical rain forests contain nearly 2,000 species of trees. Costa Rica is a diverse mixture of plant habitats and it is considered to be one of the 20 countries with greatest biodiversity in the world. Regarding screening of antifungal plants, this country has participated of the screening of antifungal plants made within OAS projects (Svetaz et al. 2010; Gaitán et al. 2011).

1.3.5.1 Canellaceae

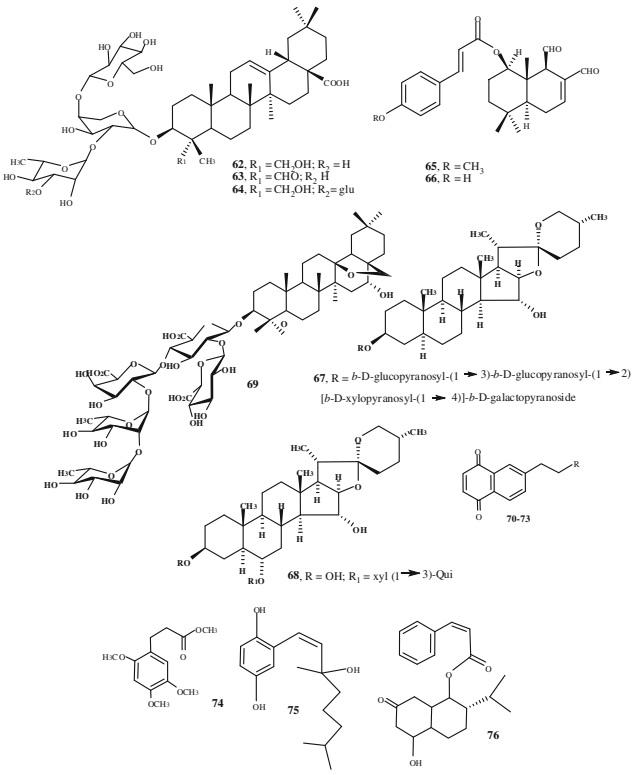
From *Pleodendron costarricense* N. Zamora, Hammel and R. Aguilar, 1-OH-4-acetyl polygodial (not shown) was isolated as the main antifungal component (Treyvand et al. 2006).

1.3.6 Guatemala

This country has performed screenings of antifungal Guatemalan plants (Cáceres et al. 1991) against human opportunistic pathogenic fungi, but the most interesting advances were the screenings made against the subcutaneous fungi *F. pedrosoi* and *S. schenckii,* which are not commonly used as targets in the antifungal panels (Gaitán et al. 2011).

1.3.6.1 Solanaceae

Antifungal *Solanum nigrescens* extracts from Guatemala led to the isolation of the steroid heterosyde cantalasaponin-3 (**67**) (He et al. 1994).



1.3.7 Mexico

1.3.7.1 Screening for Antifungal Mexican Plants

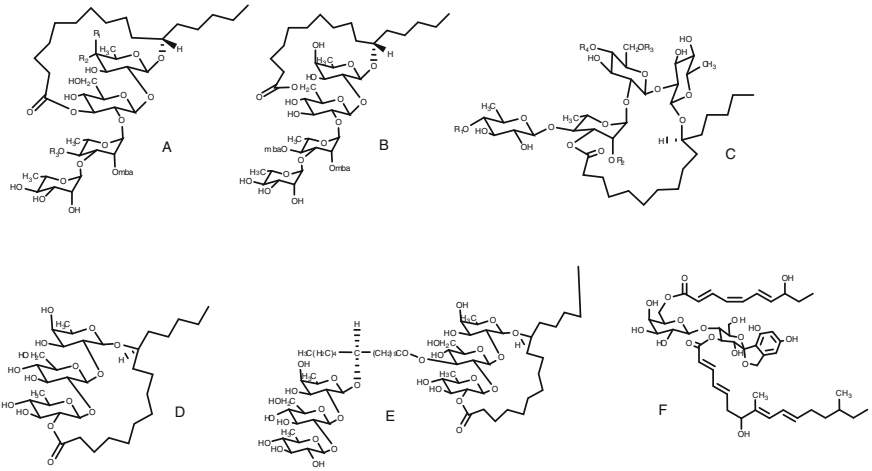
Mexico has a great plant diversity with about 40 % of endemism. Some studies performed at *random* screening of plants (Damian-Badillo et al. 2008) and others followed the ethnopharmacological approach (Navarro et al. 2003; Alanis-Garza et al. 2007).

Below, some selected antifungal compounds isolated from Mexican plants are detailed grouped by vegetal families.

1.3.7.2 Convolvulaceae

From *Ipomoea tricolor* and *Ipomoea orizabensis*, sixteen glycolipids of **A**–**F**-type macrocyclic lactones proved to be strong inhibitors of (1,3)β-glucan-synthase (Castelli et al. 2002), enzyme that catalyses the synthesis the major polymer of the

fungal cell wall, (1,3)β-glucan.



1.3.7.3 Phytolaccaceae

From Mexican folk medicinal *Phytolacca octandra* L., the fungistatic saponin serjanic acid 3-*O*-β-D-galactopyranosyl(1 → 4) − β-D-galactopyranoside (yiamoloside B) was isolated (Moreno and Rodriguez 1981).

1.3.7.4 Solanaceae

An interesting series of papers accounts for the antimycotic activity of *Solanum chrysotrichum* Schldl (common name "sosa") traditionally used in Mexico for Athlete's foot. First, the antimycotic in vitro activity was assessed against dermatophytes and *C. albicans* (Lozoya et al. 1991). Then, in a controlled and randomized double-blind clinical trial with a standardized cream containing *S. chrysotrichum* for the treatment of *tinea pedis*, the safety and effectiveness were demonstrated (Herrera-Arellano et al. 2003). Other studies achieved the isolation of five antifungal steroidal saponins from its extracts, of which **68** was the most active (Alvarez et al. 2001; Zamilpa et al. 2002). Recently, a controlled and randomized double-blind clinical trial was made on a *S. chrysotrichum* herbal medicinal product (Sc-hmp), standardized in a high percentage of **68**, for the topical treatment of vaginal infections (Herrera-Arellano et al. 2009). The study concluded that Sc-hmp exhibited similar activity than ketoconazole but with lower percentages of mycological eradication. In the same year, studies on mechanism of action on **68** showed that this compound produced structural changes such as loss of cytoplasmic membrane continuity, organelle degradation, irreversible damage to the fungal wall and cellular death of dermatophytes. Steroidal saponins,

analogues to those of *S. chrysotrichum*, were isolated from *Solanum hispidum* Pers. but they showed marginal antifungal activity (Gonzalez et al. 2004).

1.3.7.5 Theophrastaceae

Recently, the triterpenoid saponin sakurasaponin **69** was isolated from *Jaquinia flammea* Millsp. ex Mez and demonstrated to be fungicide (Garcia-Sosa et al. 2011). Although the activities were moderate, the complex structure of this saponin shows again that plants possess unpredictable structures that it is necessary not to dismiss.

1.3.8 Panama

This country has performed screenings of antifungal Panamanian plants (Rahalison et al. 1993) and was the leader of OAS screenings projects of antifungal Latin American plants (Svetaz et al. 2010; Gaitan et al. 2011).

1.3.8.1 Asteraceae

Lachnophyllum lactone, a prenylated coumarin, and a 3-methyl ether flavone (not shown) were the antifungal principles isolated from *Baccharis pedunculata* (Mill.) Cabr. The first one showed the best activity (Rahalison et al. 1995).

1.3.8.2 Boraginaceae

The meroterpenoid naphthoquinones named cordiaquinones **70**–**73** isolated from *Cordia curassavica* Roemer *et* Schultes showed high antifungal activities against *C. albicans* and *C. cucumerinum* (Ioset et al. 2000a). In turn, the phenylpropanoid derivative 1-(3′-methoxypropanoyl)-2,4,5-trimethoxybenzene **74** and the prenylated hydroquinone 2(2Z)-(3-hydroxy-3,7-dinethoxylocta-2,6-dienyl)1,4-benzenediol **75** from *Cordia alliodora* showed activity only against *C. cucumerinum* (Ioset et al. 2000b).

1.3.8.3 Clusiaceae

Three antifungal xanthones were detected as the antifungal principles of *Marila laxiflora* Rusby against *C. cucumerinum* (Ioset et al. 1998).

1.3.8.4 Myrtaceae

The essential oil from leaves of *Plinia cerrocampensis* Barrie (Myrtaceae) showed antifungal properties against dermatophytes. This species showed to be an outstanding source of α-bisabolol, a compound highly appreciated in the pharmaceutical and cosmetic industries (Vila et al. 2010).

1.3.8.5 Polygonaceae

Epicatechin gallate (not shown) was the antifungal principle of *Coccoloba dugandiana* Fern. Alonso (Maillard et al. 1987).

1.3.9 Paraguay

Portillo et al. tested three extracts of each of 14 Paraguayan plants (Portillo et al. 2001).

Below, some selected antifungal compounds isolated from Paraguayan plants are detailed grouped by vegetal families.

1.3.9.1 Asteraceae

The antifungal sesquiterpene 6-cinnamoyloxy-1-hydroxyeudesm-4-en-3-one **76** was isolated from *Vernonanthura tweediana* (Baker) H. Rob., with high activities against the clinically important fungi *C. albicans* and *C. neoformans*, (Portillo et al. 2005).

1.3.9.2 Piperaceae

From *Piper fulvescens* C. DC., three antifungal neolignans, eupomatenoid **53** and **54** and conocarpan **56** were isolated (Freixa et al. 2001).

1.3.10 Peru

Thirty-six ethanol extracts from 24 plants were screened for antifungal activities against four fungi including *C. albicans*, two dermatophytes and *S. schenckii* (Rojas et al. 2003).

1.3.10.1 Myrtaceae

From *Psidium acutangulum* DC., 3′-formyl-2′,3′,6′trihydroxychalcone (not shown) was isolated as the main antifungal compound (Wen et al. 2011).

1.3.10.2 Studies on Mechanism of Action of Compounds Isolated from Peruvian Plants

An important paper deals with natural compounds isolated from the Peruvian plants *Chlorophora tinctoria* (L.) Gaudich. ex Benth., *Paspalum conjugatum* P. J. Berg., *Symphonia globulifera* L., *Buchenavia parviflora* Ducke and *Miconia pilgeriana* Ule which showed inhibitory properties of fatty acid synthase (FAS) identified as a potential antifungal target (Li et al. 2002). The length of this chapter does not allow presenting the structures of the 13 natural inhibitors.

1.3.11 Uruguay

A screening of ten Uruguayan medicinal plants against *C. albicans* and *S. cerevisiae* was performed with agar diffusion methods (Alonso Paz et al. 1995).

1.4 Conclusions

Latin American constitutes one of the planet areas richest in biodiversity. Some efforts have been made to screen plants of the whole region and of each country but, compared to the great vegetal diversity of this region, they are insignificant. The amount of studies in each country is not correlated with its biodiversity. Regarding antifungal compounds isolated from this region, many structural types that have demonstrated antifungal properties are presented here. These studies are important starting points for the development of new antifungal drugs. However, most studies are preliminary and they begin and end with in vitro assays without comparative toxicity studies or in vivo tests. Few of them deepen the mechanisms of action and with rare exemptions, no clinical studies were carried out.

1.5 Future Trends

The antifungal compounds detected from Latin American plants deserve attention considering that some of them possess selective mechanisms of action. Development of assays in different laboratories and a close collaborative research among

Latin American countries one each other and with the whole world is highly needed and might help in the development of new natural antifungal structures from Latin American plants.

References

Agüero M, Svetaz L, Sánchez M, Luna L, Lima B, López M, Zacchino S, Palermo J, Wunderlin D, Feresin G, Tapia A (2011) Argentinean Andean propolis associated with the medicinal plant *Larrea nitida* Cav. (Zygophyllaceae). HPLC-MS and GC-MS characterization and antifungal activity. Food Chem Toxicol 49:1970–1978

Alanis-Garza B, González-González G, Salazar-Aranda R, de Torres Waksman N, Rivas-Galindo V (2007) Screening of antifungal activity of plants from the northeast of Mexico. J Ethnopharmacol 114:471–486

Alonso Paz E, Cerdeiras M, Fernandez J, Ferreira F, Moyna P, Soubes M, Vázquez A, Vero S, Zunino L (1995) Screening of Uruguayan medicinal plants for antimicrobial activities. J Ethnopharmacol 45:67–70

Álvarez L, Perez M, González J, Navarro V, Villarreal M, Olson J (2001) SC-1, antimycotic spirostan saponin from *Solanum chrysotrichum*. Planta Med 67:372–374

Alvarez S, Cortadi A, Juárez M, Petenatti E, Tomi F, Casanova J, van Baren C, Zacchino S, Vila R (2012) (-)-5,6-Dehydrocamphor from the antifungal essential oil of *Zuccagnia punctata*. Phytochemistry Lett 5:194–199

Ardao A (1993) Panamericanismo y latinoamericanismo. In: Zea L (ed) America Latina en sus ideas. Siglo XXI y UNESCO Press, Mexico, pp 157–171

Avello M, López C, Gatica C, Bustos E, Brieva A, Pastene E, Bittner M (2012) Efectos antimicrobianos de extractos de plantas chilenas de las familias Lauraceae y Atherospermataceae. Rev Cubana Plant Med 17:73–83

Baldoqui D, Kato M, Cavalheiro A, Bolzani V, Young MC, Furlan M (1999) A chromene and prenylated benzoic acid from *Piper aduncum*. Phytochemistry 51:899–902

Barnes C, Loder J (1962) Structure of polygodial, a new sesquiterpene dialdehyde from *Polygonum hydropiper* L. Austr J Chem 15:322–327

Berry P (2002) Diversidad y endemismo en los bosques neotropicales de bajura. In: Guariguata M, Catan G (eds) Ecología y conservación de bosques. Libro Universitario Regional (Eulac/GTZ), Cartago, pp 83–96

Betancur-Galvis L, Zapata B, Baena A, Bueno J, Ruiz-Nova C, Stashenko E, Mesa-Arango A (2011) Antifungal, cytotoxic and chemical analyses of essential oils of *Lippia origanoides* H.B.K. grown in Colombia. Salud UIS 43:141–148

Bovarnick A, Alpizar F, Schnell C (eds) (2010) The importance of biodiversity and ecosystems in economic growth and equity in Latin America and the Caribbean: an economic valuation of ecosystems. United Nations development programme

Braga F, Bouzada M, Fabri R, Matos M, Moreira F, Scio E, Coimbra E (2007) Antileishmanial and antifungal activity of plants used in traditional medicine in Brazil. J Ethnopharmacol 111:396–402

Brandão M, Zanetti N, Oliveira P, Grael C, Santos A, Monte-Mór R (2008) Brazilian medicinal plants described by 19th century European naturalists in the official Pharmacopoeia. J Ethnopharmacol 120:141–148

Brandão G, Kroom E, Duarte M, Braga F, de Souza Filho J, Braga de Oliveira A (2010) Antimicrobial antiviral and cytotoxic activity of extracts and constituents from Polygonum spectabile mart. Phytomedicine 17:926–929

Brian P, Curtis P, Hemming H (1949) A substance causing abnormal development of fungal hyphae produced by *Penicillium janczewskii* Zal: III. Identity of "curling factor" with griseofulvin. Trans British Mycol Soc 32:30–33

Cáceres A, López B, Giron M, Logemann H (1991) Plants used in Guatemala for the treatment of dermatophytic infections 1. Screening for antimycotic activity of 44 plant extracts. J Ethnopharmacol 31:263–276

Castelli M, Cortés J, Escalante A, Bah M, Pereda-Miranda R, Ribas J, Zacchino S (2002) In vitro inhibition of (1,3)β-glucan synthase by glycolipids from Convolvulaceous species. Planta Med 68:739–742

Castelli M, Lodeyro A, Malheiros A, Zacchino S, Roveri O (2005) Inhibition of mitochondrial ATP synthesis by polygodial, a naturally occurring dialdehyde unsaturated sesquiterpene. Biochem Pharmacol 70:82–89

Cleaves C (2001) Etnobotánica médica participativa en el Parque Nacional Lachua (Tesis). Universidad de San Carlos de Guatemala, Guatemala

Coelho de Souza G, Haas A, von Poser G, Schapoval E, Elisabetsky E (2004) Ethnopharmacological studies of antimicrobial remedies in the south of Brazil. J Ethnopharmacol 90:135–143

Correia P (1984) Dicionário de plantas úteis do brasil e das exóticas cultivadas. Instituto Brasileiro de Desenvolvimiento Forestal, Imprenta Nacional, Río de Janeiro

Cos P, Vlietink A, Vanden Berghe D, Maes L (2006) Anti-infective potential of natural products: how to develop a stronger in vitro "proof-of-concept". J Ethnopharmacol 106:290–302

Cruz M, Santos P, Barbosa A, de Mélo D, Alviano C, Antoniolli A, Alviano D, Trindade R (2007) Antifungal activity of Brazilian medicinal plants involved in popular treatment of mycoses. J Ethnopharmacol 111:409–412

Damián-Badillo L, Salgado-Garciglia R, Martínez-Muñoz R, Martinez-Pacheco M (2008) Antifungal properties of some Mexican medicinal plants. Open Nat Prod J 1:27–33

Danelutte A, Lago J, Young M, Kato M (2003) Antifungal flavanones and prenylated hydroquinones from *Piper crassinervium*. Phytochemistry 64:555–559

de Almeida Alves T, LacerdaRibeiro F, Kloos H, Zani C (2001) Polygodial, the fungitoxic component from the Brazilian medicinal plant *Polygonum punctatum*. Mem Inst Oswaldo Cruz 96:831–833

Debonsi NH, Aléci A, Kato M, Bolzani V, Young M, Cavalheiro A, Furlan M (2000) Antifungal amides from *Piper hispidum* and *Piper tuberculatum*. Phytochemistry 55:621–626

Derita M, Gattuso S, Zacchino S (2008) Occurrence of polygodial in species of *Polygonum* genus belonging to Persicaria section. Biochem System Ecol 36:55–58

Derita M, Leiva M, Zacchino S (2009) Influence of plant pñart, season of collection and content of the main active constituent on the antifungal properties of *Polygonum acuminatum* Kunth. J Ethnopharmacol 124:377–383

Derita M, Zacchino S (2011) Validation of the ethnopharmacological use of *Polygonum persicaria* for its antifungal properties. Nat Prod Comm 6:931–933

Di Liberto M, Svetaz L, Furlan R, Zacchino S, Delporte C, Novoa M, Asencio M, Cassels B (2010) Antifungal activity of saponin-rich extracts of *Phytolacca dioica* and of the sapogenins obtained through hydrolysis. Nat Prod Commun 5:1013–1018

Ekabo O, Farnsworth N, Henderson T, Mao G, Mukherjee R (1996) Antifungal and molluscicidal saponins from *Serjania salzmanniana*. J Nat Prod 59:431–435

Escalante A, Santecchia C, López S, Gattuso M, Gutiérrez Ravelo A, Delle Monache F, González M, Zacchino S (2002) Isolation of antifungal saponins from *Phytolacca tetramera*, an Argentinean species in critic risk. J Ethnopharmacol 82:29–34

Escalante A, Gattuso M, Pérez P, Zacchino S (2008) Evidence for the mechanism of action of the antifungal phytolaccoside B isolated from *Phytolacca tetramera* Hauman. J Nat Prod 71:1720–1725

Espinel-Ingroff A (2009) Novel antifungal agents, targets or therapeutic strategies for the treatment of invasive fungal diseases: a review of the literature (2005–2009). Rev Iberoamer Micol 26:15–22

Estévez Y, Castillo D, Tangoa Pisango M, Arévalo J, Rojas R, Alban J, Deharo E, Bourdy G, Sauvain M (2007) Evaluation of the leishmanicidal activity of plants used by Peruvian Chayahuita ethnic group. J Ethnopharmacol 114:254–259

Fenner R, Sortino M, Kuze Rates S, Dall′Agnol R, Ferraz A, Bernardi A, Albring D, Nor C, von Poser G, Schapoval E, Zacchino S (2005) Antifungal activity of some Brazilian *Hypericum* species. Phytomedicine 12:136–140

Freitas G, Saga Kitamura R, Lago J, Young M, Guimaraes E, Kato M (2009) Caldensinic acid, a prenylated benzoic acid from *Piper caldense*. Phytochemistry Lett 2:119–122

Freixa B, Vila R, Ferro E, Adzet T, Cañigueral S (2001) Antifungal principles from *Piper fulvescens*. Planta Med 67:873–875

Freixa B, Vila R, Vargas L, Lozano N, Adzet T, Cañigueral S (1998) Screening for antifungal activity of nineteen Latin American plants. Phytother Res 12:427–430

Frost D, Brandt K, Cugier D, Goldman R (1995) A whole cell *Candida albicans* assay for the detection of inhibitors towards fungal cell wall synthesis and assembly. J Antibiot 48:306–310

Fukuda D, Natatsukasa W, Yao R, Hunt A, Gordee R, Zeckner D, Mynderse J (1991) A45507, a complex of fungal cell wall inhibitors produced by a mold. 31st ICAAC meeting (Chicago, Il, USA) poster #211

Gaitán I, Paz A, Zacchino S, Tamayo G, Giménez A, Pinzón R, Cáceres A, Gupta M (2011) Subcutaneous antifungal screening of Latin American plant extracts against *Sporothrix schenckii* and *Fonsecaea pedrosoi*. Pharm Biol 49:907–919

Garcia-Sosa K, Sanchez-Medina A, Álvarez S, Zacchino S, Veitch N, Simá-Polanco P, Peña-Rodríguez L (2011) Antifungal activity of sakurasaponin from the root extracts of *Jacquinia flammea*. Nat Prod Res 25:1185–1189

Gold W, Stout H, Pagano J, Donovic R (1955) Amphotericins A and B, antifungal antibiotics produced by a streptomycete. I. In vitro studies. Antib Ann 3:579–586

Goleniowski M, Bongiovanni G, Palacio L, Núñez C, Cantero J (2006) Medicinal plants from the "Sierra de Comechingones", Argentina. J Ethnopharmacol 107:324–341

Gonzalez M, Zamilpa A, Marquina S, Navarro V, Alvarez L (2004) Antimycotic spirostanol saponins from *Solanum hispidum* leaves and their structure-activity relationships. J Nat Prod 67:938–941

Gupta M (1995) 270 Plantas medicinales Iberoamericanas. CYTED-Convenio Andrés Bello (ed) Bogotá

Gupta M (2008) Plantas medicinales Iberoamericanas. Convenio Andrés Bello (ed) Bogotá

Gutkind G, Martino V, Graña N, Coussio J, de Torres R (1981) Screening of South American plants for biological activities. Fitoterapia 52:213–218

Hammond PM (1995) The record to date: described species. In: Heywood V, Watsin R (eds) Global biodiversity assessment. Cambridge University Press, Cambridge, pp 113–138

He X, Mocek U, Floss H, Caceres A, Giron L, Buckley H, Cooney G, Manns J, Wilson B (1994) An antifungal compound from *Solanum nigrescens*. J Ethnopharmacol 43:173–177

Hernández M, Abedini W and Delucchi G (1998) Estrategias para la conservación de Phytolacca tetramera Hauman (Phytolaccaceae), especie endémica de la Provincia de Buenos Aires; Abstract. XXVI Jornadas Argentinas de Botánica, Rio Cuarto (Córdoba), Cartel N° 307

Herrera-Arellano A, Jiménez-Ferrer E, Zamilpa A, Martínez-Rivera M, Rodríguez-Tovar A, Herrera-Alvarez S, Salas-Andonegui M, Nava-Xalpa M, Méndez-Salas A, Tortoriello J (2009) Exploratory study on the clinical and mycological effectiveness of herbal medicinal product from *Solanum chrysotrichum* in patients with *Candida* yeast-associated vaginal infections. Planta Med 75:466–471

Herrera-Arellano A, Rodríguez-Soberanes A, Martínez-Rivera M, Martínez-Cruz E, Zamilpa A, Álvarez L, Tortoriello J (2003) Effectiveness and tolerability of a standardized phytodrug derived from *Solanum chrysotrichum* on tinea pedis: a controlled and randomized clinical trial. Planta Med 69:390–395

Ioset J, Marston A, Gupta M, Hostettmann K (1998) Antifungal xanthones from roots of *Marila laxiflora*. Pharm Biol 36:103–106

Ioset J, Marston A, Gupta M, Hostettmann K (2000a) Antifungal and larvicidal cordial quinones from the roots of *Cordia curassavica*. Phytochemistry 53:613–617

Ioset J, Marston A, Gupta M, Hostettmann K (2000b) Antifungal and larvicidal compounds from the root bark of *Cordia alliodora*. J Nat Prod 63:424–426

Koroishi A, Foss S, Garcia Cortez D, Ueda-Nakamura T, Nakamura C, Dias Filho B (2008) In vitro antifungal activity of extracts and neolignans from *Piper regnelli* against dermatophytes. J Ethnopharmacol 117:270–277

Kurdelas R, Tapia A, Lima B, Feresin G, González Sierra M, Rodríguez M, Zacchino S, Enriz R, Freile M (2010) Antifungal activity of extracts and prenylated coumarins isolated from Baccharis darwinii (Asteraceae). Molecules 15:4898–4907

Kuster R, Arnold N, Wessjohann L (2009) Antifungal flavonoids from *Tibouchina grandifolia*. Biochem Syst Ecol 37:63–65

Lago J, Chaves M, Ayres M, Agripino D, Young M (2007) Evaluation of antifungal DNA-damaging activities of alkaloids from branches of *Porcelia macrocarpa*. Planta Med 73:292–295

Lago J, Chen A, Young M, Guimaraes E, de Oliveira A, Kato M (2009) Prenylated benzoic acid derivatives from *Piper aduncum* L. and *P. hostmannianum* C. DC. (Piperaceae). Phytochem Lett 2:96–98

Lee S, Lee J, Lunde C, Kubo I (1999) In vitro antifungal susceptibilities of *Candida albicans* to polygodial, a sesquiterpene dialdehyde. Planta Med 65:204–208

Li X, Josji A, ElSohly H, Khan S, Jacob M, Zhang Z, Kahn I, Ferreira D, Walker L, Broedel S, Raulli R, Cihlar R (2002) Fatty acid synthase inhibitors from plants: isolation, structure elucidation and SAR studies. J Nat Prod 65:1909–1914

Lima B, Agüero M, Zygadlo J, Tapia A, Solis C, Rojas de Arias A, Yaluff G, Zacchino S, Feresin G, Schmeda G (2009) Antimicrobial activity of extracts, essential oil and metabolites obtained from *Tagetes mendocina*. J Chil Chem Soc 54:68–72

Lima B, López S, Luna L, Agüero M, Aragón L, Tapia A, Zacchino S, López M, Zygadlo J, Feresin G (2011) Essential oils of medicinal plants from the central Andes of Argentina: chemical composition and antifungal., antibacterial and insect repellent activities. Chem-Biodivers 8:924–936

Lima B, Sánchez M, Luna L, Agüero M, Zacchino S, Filippa E, Palermo J, Tapia A, Feresin G (2012) Antimicrobial and antioxidant activities of *Gentianella multicaulis* collected on the Andean slopes of San Juan province. Z Naturforsch C 67:29–38

Lima E, Morais V, Gomes S, Cechinel Filho V, Miguel O, Yunes R (1995) Preliminary evaluation of antifungal activity of xanthoxyline. Acta Farm Bonaer 14:213–216

Lopes N, Kato M, Yoshida M (1999) Antifungal constituents from roots of *Virola surinamensis*. Phytochemistry 51:29–33

López S, Furlan R, Zacchino S (2011) Detection of antifungal compounds in *Polygonum ferrugineum* Wedd., extracts by bioassay-guided fractionation- Some evidences of their mode of action. J Ethnopharmacol 138:633–636

Lozoya X, Navarro V, García M, Zurita M (1991) *Solanum chrysotrichum* (Schidl.) a plant used in Mexico for the treatment of skin mycoses. J Ethnopharmacol 36:127–132

Maillard M, Gupta M, Hostettmann K (1987) Antifungal activity of epicatechin gallate from *Coccoloba dugandiana*. Planta Med 65:781–782

Malheiros A, Cechinel Filho V, Schmitt C, Yunes R, Escalante A, Svetaz L, Zacchino S, Delle Monache F (2005) Antifungal activity of drimane sesquiterpenes from *Drimys brasiliensis* using bioassay-guided fractionation. J Pharm Pharmaceut Sci 8:335–339

Mathew B, Nath M (2009) Recent approaches to antifungal therapy for invasive mycoses. ChemMedChem 4:310–323

McCallion R, Cole A, Walker J, Blunt J, Munro M (1982) Antibiotic substances from New Zealand plants 2. Polygodial, an anti-*Candida* agent from *Pseudowintera colorata*. Planta Med 44:134–138

Mesa-Arango A, Montiel J, Martínez C, Zapata C, Pino N, Bueno J, Stashenko E (2007) Actividad in vitro anti-*Candida* y anti-*Aspergillus* de aceites esenciales de plantas de la familia Piperaceae. Scientia et Technica XIII:247–249

Mesa-Arango A, Montiel J, Betancur-Galvis L, Bueno J, Baena A, Durán D, Martínez J, Stashenko E (2008) Antifungal activity and chemical composition of the essential oils of Lippia alba (Miller) N.E Brown, grown in different regions of Colombia. J Essent Oil Res 163:337–344

Mesa-Arango A, Montiel-Ramos J, Zapata B, Durán C, Betancur-Galvis L, Stashenko E (2009) Citral and carvone chemotypes from the essential oils of Colombian *Lippia alba* (Mill.)N.E. Brown: composition, cytotoxicity and antifungal activity. Mem Inst Oswaldo Cruz 104:878–884

Morales G, Paredes A, Sierra P, Loyola L (2008) Antimicrobial activity of three species of *Baccharis* used in the traditional medicine of Northern of Chile. Molecules 13:790–794

Moreno M, Rodriguez V (1981) Yiamoloside B a fungistatic saponin of *Phytolacca octandra*. Phytochemistry 20:1446–1447

Muschietti L, Derita M, Sulsen V, Muñoz J, Ferraro G, Zacchino S, Martino V (2005) In vitro antifungal assay of traditional Argentine medicinal plants. J Ethnopharmacol 102:232–238

Navarro V, Gonzalez A, Fuentes M, Aviles M, Rios M, Zepeda G, Rojas M (2003) Antifungal activities of nine traditional Mexican plants. J Ethnopharmacol 87:85–88

Odds F (2005) Genomics, molecular targets and the discovery of antifungal drugs. Rev Iberoam Micol 22:229–237

Pacciaroni A, Gette M, Derita M, Ariza-Espinar L, Gil R, Zacchino S, Silva G (2008) Antifungal activity of *Heterothalamus alienus* metabolites. Phytother Res 22:524–528

Peláez F, Cabello A, Platas G, Diez M, González del Val A, Martán I, Vicente F, Bills G, Giacobbe R, Schwartz R, Onishi J, Meinz M, Abbruzzo G, Flattery A, Kong L, Kurtz M (2000) The discovery of enfumafungin, a novel antifungal compound produced by an endophytic *Hormonema* spp biological activity and taxonomy of the producing organisms. System Appl Microbiol 23:333–343

Peralta M, Calise M, Fornari M, Ortega M, Diez R, Cabrera J, Pérez C (2012) A prenylated flavanone from *Dalea elegans* inhibits rhodamine 6G efflux and reverses fluconazole resistance in *Candida albicans*. Planta Med 78:981–987

Pessini G, Dias Filho B, Nakamura C, Garcia Cortez D (2005) Antifungal activity of the extracts and neolignans from *Piper regnelli* (Miq.) C. DC. var. *pallescens* (C.DC.) Yunck. J Braz Chem Soc 16:1130–1133

Petenatti E, Gette M, Derita M, Petenatti M, Solis C, Zuljan F, Del Vitto L, Zacchino S (2008) Importance of the ethnomedical information for the detection of antifungal properties in plant extracts from the Argentine flora. In: Martino V, Muschietti L (eds) South American medicinal plants as potential source of bioactive compounds. Transworld Research Network, Kerala, pp 15–38

Portillo A, Vila R, Freixa B, Adzet T, Cañigueral S (2001) Antifungal activity of Paraguayan plants used in traditional medicine. J Ethnopharmacol 76:93–98

Portillo A, Vila R, Freixa B, Ferro E, Parella T, Casanova J, Cañigueral S (2005) Antifungal sesquiterpene from the root of *Vernonanthura tweediana*. J Ethnopharmacol 97:49–52

Quiroga E, Sampietro A, Vattuone M (2001) Screening antifungal activities of selected medicinal plants. J Ethnopharmacol 74:89–96

Quiroga E, Sampietro D, Sgariglia M, Soberón J, Vattuone M (2008) Antimycotic activity of 5′-prenylisoflvanones of the plant *Geoffroea decorticans* against *Aspergillus* species. Int J Food Microbiol 132:42–46

Rahalison L, Benathan M, Monod M, Frenk E, Gupta M, Solis PN, Fuzzati N, Hosttetmann K (1995) Antifungal principles of *Baccharis pedunculata*. Planta Med 61:360–362

Rahalison L, Hamburger M, Hostetmann K, Monod M, Frenk E, Gupta M, Santana A, Correa M, Gonzalez A (1993) Screening for antifungal activity of Panamanian plants. Pharm Biol 31:68–76

Ríos J, Recio M (2005) Medicinal plants and antimicrobial activity. J Ethnopharmacol 100:80–84

Rojas R, Bustamanrte B, Bauer J, Fernández I, Albán J, Lock O (2003) Antimicrobial activity of selected Peruvian medicinal plants. J Ethnopharmacol 88:199–204

Rouhi M (2003) Rediscovering natural products. Chem Engin News 81:77–91

Sanz-Biset J, Campos-de-la-Cruz J, Epiquién-Rivera M, Cañigueral S (2009) A first survey on the medicinal plants of the Chazuta valley (Peruvian Amazon). J Ethnopharmacol 122:333–362

Sartori M, Pretto J, Cruz A, Brescianio F, Yunes R, Sortino M, Zacchino S, Cechinel Filho V (2003) Antifungal activity of fractions and two pure compounds of flowers from *Wedelia paludosa* (*Acmela brasiliensis*) Asteraceae. Pharmazie 58:567–569

Scarpa G (2004) Medicinal plants used by the criollos of northwestern Argentine Chaco. J Ethnopharmacol 91:115–135

Silva I Jr, Cechinel Filho V, Zacchino S, da S Lima C, de Oliveira Martins D (2008) Antimicrobial screening of some medicinal plants from Mato Grosso Cerrado. Braz J Pharmacogn 19:242–248

Silva I Jr, Raimondi M, Zacchino S, Cechinel Filho V, Noldin V, Rao V, Silva R, Lima J, de Oliveira Martins D (2010) Evaluation of the antifungal activity and mode of action of *Lafoensia pacari* St Hil., Lythraceae, stem-bark extracts, fractions and ellagic acid. Braz J Pharmacogn 20:422–428

Stein A, Alvarez S, Avancini C, Zacchino S, von Poser G (2006) Antifungal activity of some coumarins obtained from species of *Pterocaulon* (Asteraceae). J Ethnopharmacol 107:95–98

Svetaz L, Zuljan F, Derita M, Petenatti E, Tamayo G, Cáceres A, Cechinel Filho V, Giménez A, Pinzón R, Zacchino S, Gupta M (2010) Value of the ethnomedical information for the discovery of plants with antifungal properties. A survey among seven Latin American countries. J Ethnopharmacol 127:137–158

Teixeira M, Figueira G, Sartoratto A, Garcia Reder V, Delarmelina C (2005) Anti-*Candida* activity of Brazilian medicinal plants. J Ethnopharmacol 97:305–311

Treyvand V, Petit P, Ta C, Nuñez R, Sanchez-Vindas P, Poveda L, Smith M, Arnason J, Durst T (2006) Phytochemistry and antifungal properties of the newly discovered tree *Pleodendron costaricense*. J Nat Prod 69:1005–1009

Vasques da Silva R, Debonsi NH, Kato M, Bolzani V, Méda C, Young M, Furlan M (2002) Antifungal amides from *Piper arboreum* and *Piper tuberculatum*. Phytochemistry 59:521–527

Vila R, Santana A, Pérez-Roses R, Valderrama A, Castelli M, Mendonca S, Zacchino S, Gupta M, Cañigueral S (2010) Composition and biological activity of the essential oil from leaves of *Plinia cerrocampanensis,* a new source of α-bisabolol. Biores Technol 101:2510–2514

Weitzman I, Summerbell R (1995) The dermatophytes. Clin Microbiol Rev 8:240–259

Wen L, Haddad M, Fernández I, Espinoza G, Ruiz C, Neyra E, Bustamante B, Rojas R (2011) Actividad antifúngica de cuatro plantas usadas en la medicina peruana. Aislamiento de 3′-formil-2′4′6′-trihidroxichalcona, principio activo de *Psidium acutangulum*. Rev Soc Quim Peru 77:199–204

Zacchino S, Rodriguez G, Pezzenati G, Orellana G (1997) In vitro evaluation of antifungal properties of 8.O.4′-neolignans. J Nat Prod 60:659–662

Zacchino S, Santecchia C, López S, Muñoz J, Cruañes A, Vivot E, Cruañes M, Salinas A, Ruiz R, Ruiz S (1998) In vitro antifungal evaluation and studies on mode of action of eight selected species from the Argentine flora. Phytomed 5:389–395

Zamilpa A, Tortoriello J, Navarro V, Delgado G, Alvarez L (2002) Five new steroidal saponins from *S. chrysotrichum* leaves and their antimycotic activity. J Nat Prod 65:1815–1819

Zapata B, Durán C, Stashenko E, Betancur-Galvis L, Mesa-Arango A (2010) Actividad antimicótica y citotóxica de aceites esenciales de plantas de la familia Asteraceae. Rev Iberoam Micol 27:101–103

Chapter 2
Antifungal Plants of Iran: An Insight into Ecology, Chemistry, and Molecular Biology

Mehdi Razzaghi-Abyaneh, Masoomeh Shams-Ghahfarokhi and Mahendra Rai

Abstract Worldwide occurrence of fungal infections has been dramatically increased in recent years due to a continuous increase in immunosuppressive conditions like AIDS, organ transplantation and hematologic malignancies. Fungal infections are major concerns in Iran with an increasing numbers of new reports from superficial to deep hospital-acquired infections every year. Although there are no comprehensive data on the real incidence of fungal infections, especially systemic ones in Iran, about 50 % of suspected individuals referred to our laboratory (Mycology Department of the Pasteur Institute of Iran) were found to have dermatophytosis, candidiasis, and pityriasis versicolor (Sadeghi et al. 2011). Plants are rich sources of beneficial secondary metabolites which are attractive as flavors, fragrances, pesticides, pharmaceuticals, and antimicrobials. Increasing trends of health organizations and pharmaceutical industries to use plants as safe and effective alternative sources of synthetic antifungals are due to major problems of slow growing and high costs of synthetic pharmaceutics, their life-threatening side effects, rapid increase in new fungal infections, and dramatic emergence of multidrug-resistant fungal pathogens. Interestingly, antifungal drug discovery from medicinal plants is a rapidly growing industry worldwide (WHO 2002). World trade of medicinal plants is now more than 43 billion dollars and has been predicted to reach to 5 trillion dollars in 2050. It has been estimated that around 7,500–8,000 plant species are growing in Iran of which only 130 species have been routinely used as anti-infective drugs in traditional medicine (Rechinger 1982). Iran's contribution to this market is about 60 million dollars, which

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increases every year (Noorhosseini Niyaki et al. 2011). This chapter highlights the current status of antifungal plant flora of Iran regarding their ecology, biochemistry, and molecular biology. Special attention will be made to effective plant components responsible for antifungal properties.

Abbreviations

Albugo	*A. candida*
Aspergillus	*A. niger*, *A. flavus*, *A. parasiticus*, *A. fumigatus*
Botrytis	*B. cinerea*
Candida	*C. albicans*, *C. glabrata*, *C. tropicalis, C. parapsilosis*
Cladosporium	*C. cucumerinum*
Cochliobolus	*C. sativus*
Corynespora	*C. cassilicola*
Epidermophyton	*E. floccosum*
Fusarium	*F. oxysporum* f. sp. *radicis-cucumerinum*, *F. solani*, *F. oxysporum*, *F. poae*, *F. equiceti, F. verticillioides*
Macrophomina	*M. phaseolina*
Malassezia	*M. furfur*, *M. globosa*, *M. obtusa*
Microsporum	*M. canis*, *M. gypseum*
Phytophthora	*P. drechsleri*
Rhizoctonia	*R. solani*
Saccharomyces	*S. cereviciae*
Trichoderma	*T. harzianum*
Trichophyton	*T. mentagrophytes*, *T. rubrum*
Verticillium	*V. dahliae*

2.1 Introduction

Despite tremendous success in development of medical sciences in recent years, infectious diseases remain the second leading cause of human death worldwide (WHO 2002). Fungi comprise a major part of biodiversity, second to insects owing to have an estimated number of 1.5 million species on our planet. Among 100,000 known fungal species, around 400 species are known as human and animal pathogens. Although fungal diseases are not important as much as bacterial and viral infections, however, they are increasing in number and severity due to easy airborne distribution of fungal spores as infective propagules and unique adaptability to various environmental conditions. Increasing resistance of fungal pathogens especially life-threatening genera, that is, *Aspergillus* and *Candida* to synthetic antibiotics urged pharmaceutical industries all over the world to search for safer and more effective alternatives from natural sources. Among beneficial

biodiversity, plants are in the first line of such investigations due to having a large number of antifungal metabolites with unique structural diversity (Balunas and Kinghorn 2005). Plant metabolites have advantages to microbial metabolites regarding their low molecular weights, which makes them suitable for formulating as antifungal drugs (Cowan 1999).

The history of using plants as healing agents dates back to around 60,000 years ago when the Neanderthals, landed in present-day Iraq, used hollyhock (*Alcea rosea* L.) as a remedy (Stockwell 1988). Plants are important not only as essential part of the ecosystem as food but also for being unique sources of natural antimicrobials which makes them interesting as alternatives of synthetic antimicrobials (Clark 1996; Cowan 1999; Bakkali et al. 2008; Razzaghi-Abyaneh et al. 2010; Abdallah 2011; Razzaghi-Abyaneh and Shams-Ghahfarokhi 2011). It has been estimated that around 25,000–50,000 plant species exist in our planet have ethnobotanical importance (Borris 1996).

Iran with an area of 1,648,195 million km^2 is the eighteenth largest country in the world located in three spheres of Asia (West, Central, and South) in Middle East. It has about 33 % of cultivable land, 14 million ha pasture, 60 million ha steppes, and 16 million ha deserts. Because of particular climatic significance owing to possess 11 climates out of 13 world climates, Iran is a rich source of medicinal plants, and some of them were employed in traditional medicine for centuries (Sharafzadeh and Alizadeh 2012).

The first description of use of medicinal plants as remedies in Iran dates back to the earliest known civilizations, the Sumerians in 3000 B.C. (Price 2001). It seems that the earliest and the oldest production in prose of the Neo-Persian literature on pharmacology is the "kitabulabnyat and haqa'iq-uladviyat" or "Book of the Foundations of the True Properties of the Remedies" written about 970 A.D. by the Persian Physician Abu Mansur. The book has been examined by R. Seligmann from the oldest existent Persian manuscript of Vienna dated 1055. During 200–460 BC, the famous university and the hospital at Jundishapur were established. It was a start to the golden age of herbal-based medicine in Iran which reached to the top by the famous scientists Zakariya-Al-Razi (Rhazes 865–925), Al-Biruni (973–1048), and Abu Ali Sina (Avicenna 980–1037). *The Canon of Medicine* (Al-Qanoon fi al-Tibb, The Laws of Medicine), written by Avicenna, described the ethnobotanical and therapeutic effects of about 811 medicinal plants. It was a standard medical text in Europe and the Islamic world until the eighteenth century and played a crucial role in European Renaissance (Price 2001).

In a Tehran publication of 1874 written in French by Professor J.L. Schlimmer of the Polytechnic College of Teheran entitled "Terminologie Médico-Pharmaceutique et Anthropologique Francaise-Persane," a full list of medicinal plants of Iran was published. In 1890, Dr. J.E.T. Aitchison botanically explored portions of Iran and the neighboring regions in "Notes on the Products of Western Afghanistan and of North–Eastern Persia" published in Edinburgh. During 1929–1958, five collections of medicinal plants of Iran were published which comprised about 200,000 herbarium specimens from different parts of Iran (Pasrsa 1959a, b, c; 1960). Three of these collections were published in "Useful plants and drugs of

Iran and Iraq" by David Hooper, 1937. Other two collections were originated from the work of Ahmad Pasha, founder and leader of the Museum of Natural History of Teheran, during 1946–1958, which published under the names "Flore de I'Iran" and "Medicinal plants and drugs of plant origin in Iran; parts I–IV."

We represent here current data about antifungal properties of essential oils and extracts prepared from various parts (leaves, stems, roots, flowers, seeds, shoots) of indigenous plants of Iran.

2.2 Medicinal Plants with Antifungal Properties

A total of 83 plant species from flora of Iran distributed into 28 families were found in various surveys to have antifungal properties. Table 2.1 illustrates detailed data about these plants and their bioactive metabolites inhibitory to a wide array of fungal pathogens. The following sections will discuss the antifungal properties of the most effective plants listed in Table 2.1.

2.2.1 Matricaria chamomilla

Matricaria chamomilla L. (syn: *M. recutita* L.; German chamomile) resides in the Asteraceae (Compositae) family and is the most important species within the genus, and it is endemic to Iran as a carminative, stimulant, and febrifuge remedy. It has a long history of application in herbal medicine as carminative and anti-inflammatory agent. Camomile tea prepared from the daisies is given to relieve intercostal neuralgia. An infusion of the drug is prescribed for dysentery.

Essential oil of plant flowers has shown antifungal properties by inhibiting the growth and conidiogenesis of *Aspergillus niger* (Tolouee et al. 2010). Complementary electron microscopic studies revealed that the plant oil inhibited *A. niger* growth by affecting fungal cell membrane, probably through interaction with ergosterol biosynthesis (Fig. 2.1). A monocyclic sesquiterpene alcohol named α-bisabolol, the main component of plant oil, is accounted for antifungal properties because of structural similarities to zymosterol, an important intermediate in ergosterol biosynthesis pathway (Pauli 2005, 2006).

2.2.2 Stachys Inflata

The genus *Stachys* (Lamiaceae) is represented by 270 species with 34 species found in Iran of which 13 species are endemic (Zargari 1992; Ghahreman 1995). *Stachys* species are well known to have chemically diverse compounds including phenylethanoid glycosides, terpenoids, steroids, diterpenes, and flavonoids.

Table 2.1 General characteristics of antifungal plant flora of Iran

Family	Species	Common name (place of collection)	Part used	Effective component (s)	Inhibitory concentration	Affected fungi	Reference
Alliaceae	*Allium cepa* L.	Onion	Bulb	Aqueous extract	0.125–2 mg/ml-MIC_{50}	*M. furfur*	Shams-Ghahfarokhi et al. (2003), (2004), (2006)
						C. albicans	
					2–8 mg/ml-MIC_{90}		
						C. glabrata	
						C. tropicalis	
						C. parapsilosis	
	Allium sativum L.	Garlic			0.0039–0.0312 mg/ml-MIC_{50}	*T. mentagrophytes*	
						T. rubrum	
						M. canis	
						M. gypseum	
						E. floccosum	
					0.0156–0.125 mg/ml-MIC_{90}		
Anacardiaceae	*Rhus coriaria*	Sumac (Kermanshsh)	Shoot	Methanolic extract	50 ppm-MFC	*A. candida*	Omranpour et al. (2011)
Apiaceae	*Conium maculatum* L.	Hemlock	Leaves	Essential oil	90 % inhibition at 1,000 μg/ml	*A. parasiticus*	Razzaghi-Abyaneh et al. (2009)
	Ferula gummosa	Galbanum	Aerial parts	Essential oil (ß-pinene as the main constituent; 54.4 %)	1,815 μg/ml-IC_{50}	*A. parasiticus*	Alinezhad et al. (2011)
	Diplotaenia damavandica Mozaff. Ex Hedge and Lamond	Kozal	Leaves	Essential oil		*C. albicans*	Eftekhar et al. (2005)
						A. niger	
						S. cereviciae	
				Oil constituents tested;	3.6–7.2 mg/ml-MICs		
				– Dillapiol	>64.3 mM		
				– Terpinolene	6.6–13.2 mM		
				– γ-terpinene	55.1->110.1 mM		
				– *p*-cymene	>55.9 mM-MICs		

Table 2.1 (continued)

Family	Species	Common name (place of collection)	Part used	Effective component (s)	Inhibitory concentration	Affected fungi	Reference
	Cuminum cyminum	Cumin Ziree in Percian	Aerial parts	Essential oil (α-pinene, limonene and 1,8-cineol as the main constituents; 30, 21 and 18.5 %)	30 µl/disk	*M. furfur*	Naeini et al. (2011)
						M. globosa	
						M. obtusa	
				Essential oil	59–578 µg/ml-MICs	*F. solani*	Naeini et al. (2010)
						F. oxysporum	
						F. verticillioides	
						F. poae	
						F. equiseti	
				Essential oil	280 µg/ml-MIC	*C. albicans*	Naeini et al. (2009)
	Heracleum persicum Desf. Ex Fisch.	Persian cow-parsnip	Aerial parts	Essential oil	70–4,500 µg/ml-MICs	*F. solani*	Naeini et al. (2010)
						F. oxysporum	
		Golpar in Percian				*F. verticillioides*	
						F. poae	
						F. equiseti	
				Essential oil (*trans*-anethole as the main constituent; 25.0 %)	2 mg/disk	*A. niger*	Firuzi et al. (2010)
					–Inhibition zone of ~7 mm	*C. albicans*	
				Essential oil	1,100 µg/ml-MIC	*C. albicans*	Naeini et al. (2009)
			Seeds	Ethyl acetate extract	370 µg/ml-IC_{50}	*A. parasiticus*	Alinezhad et al. (2011)
	Heracleum pastinacifolium K. Koch	Hogwood	Aerial parts	Essential oil (myristicin as the main constituent; 53.6 %)	2 mg/disk	*A. niger*	Firuzi et al. (2010)
					–Inhibition zone of ~7 mm	*C. albicans*	

(continued)

Table 2.1 (continued)

Family	Species	Common name (place of collection)	Part used	Effective component (s)	Inhibitory concentration	Affected fungi	Reference
	Heracleum rechingeri Manden	Hogwood		Essential oil (hexyl butanoate as the main constituent; 29.7%)			
	Heracleum transcaucasicum Manden	Hogwood		Essential oil (elemicin as the main constituent; 41.1 %)			
	Foeniculum vulgare	Fennel	Roots	Essential oil (dillapiol as the main constituent; 90.1 %)	700 µg/ml-IC_{50}	*A. parasiticus*	Alinezhad et al. (2011)
			Flowers	Essential oil (*trans*-anethole as the main constituent; 68.4 %)	~70 % inhibition at 2,000 µg/ml		
			Aerial parts	Essential oil	64–1,232 µg/ml-MICs	*F. solani* *F. oxysporum* *F. verticillioides* *F. poae* *F. equiseti*	Naeini et al. (2010)
				Essential oil	300 µg/ml	*C. albicans*	Naeini et al. (2009)
	Foeniculum miller	Hay	Leaves	Essential oil	~15.0 % inhibition at 1,000 µg/ml	*A. parasiticus*	Razzaghi-Abyaneh et al. (2009)
	Semenovia tragioides Boiss.	- (Kashan)	Aerial parts	Essential oil (lavandulyl acetate, geranyl acetate and *trans*-ocimene as the main constituents; 25.5, 12.5 and 8.8 %) Methanolic extract	5 mg/ml-MIC	*C. albicans (no effect on A. niger)*	Bamoniri et al. (2010)
	Pimpinella anisum L.	Anise	Aerial parts	Essential oil	300 µg/ml-MIC	*C. albicans*	Naeini et al. (2009)
			Seeds	Methanolic extract	16–64 mg/ml-MICs	*C. albicans* *M. canis*	Yazdani et al. (2009)

(continued)

Table 2.1 (continued)

Family	Species	Common name (place of collection)	Part used	Effective component (s)	Inhibitory concentration	Affected fungi	Reference
						T. mentagrophytes	
						E. floccosum	
	Trechyspermum copticum	Ajowan	Fruits	Essential oil (thymol, γ-terpinene and O-cymene as the main constituents: 45.9, 20.6 and 19.0 %)	0.25–0.5 µg/ml-MICs	*C. albicans*	Mahboubi and Kazempour (2011)
						C. glabrata	
						A. niger	
					0.25–1.0 µg/ml-MLCs		
						A. flavus	
						A. parasiticus	
	Trachyspermum ammi Spraque ex Turrill.	Ajowan	Seeds	Essential oil	10–55 µg/disk	*C. albicans*	Ghasemi-Pirbalouti et al. (2009)
					Inhibition zone of 18–21 mm		
	Bunium persicum Boiss.	Kala jeera	Fruits	Essential oil	8–256 µg/ml	*A. niger*	Ghasemi-Pirbalouti et al. (2011)
						A. flavus	
						A. fumigatus	
Asteraceae	*Centaurea behen* Linn.	Safed behman	Whole plant	Aqueous and methanolic extracts	5 mg/disk	*R. solani*	Bahraminejad et al. (2011)
						F. oxysporum	
						Cochliobolus sativus	
					Inhibition zone of 6.2–15.2 mm		
	Achillea millefolium subsp. *elborsensis*	Yarrow Plumajillo (Alborz)	Flowers	Essential oil (chamazulene as the main constituent; 48.9 %)	580 µg/ml-IC_{50}	*A. parasiticus*	Alinezhad et al. (2011)
	Matricaria chamomilla L.	German chamomile	Flowers	Essential oil (α-bisabolol as the main constituent; 56.86 %)	300 µg/ml-IC_{50}	*A. niger*	Tolouee et al. (2010)

(continued)

Table 2.1 (continued)

Family	Species	Common name (place of collection)	Part used	Effective component (s)	Inhibitory concentration	Affected fungi	Reference
	Artemisia scoparia Waldst. and Kitam.	Redstem wormwood	Aerial parts	Methanolic extract	80 mg/ml, 0.2 ml/cup inhibition zone of 13.6 mm	*C. albicans*	Ramezani et al. (2004)
	Artemisia dracunculus	Tarragon	Leaves	Essential oil (terpinolene, *trans*-ocimene and *trans*-anethole as the main constituents; 12.4, 20.6 and 21.2 %)	70.1 % inhibition at 1,000 µg/ml	*A. parasiticus*	Razzaghi-Abyaneh et al. (2009)
	Artemisia sieberi Besser	Wormwood (Ghom-Markazi)	Aerial parts	Essential oil (β-thujone, camphor and α-thujone as the main constituents; 23.0, 19.5 and 15.0 %)	37.4–4,781.3 µg/ml-MICs	*C. glabrata*	Khosravi et al. (2011)
					597.6–4,781.3 µg/ml-MFCs		
				Essential oil	1,100 µg/ml-MIC	*C. albicans*	Naeini et al. (2009)
	Artemisia kermanensis Podl.	Wormwood (Kerman)	Aerial parts	Essential oil	10–55 µg/disk	*C. albicans*	Ghasemi-Pirbalouti et al. (2009)
					Inhibition zone of 7–9 mm		
	Tanacetum polycephalum	Tansy (Chaharmahal va Bakhtiari)	Flowers		10–55 µg/disk		
					Inhibition zone of 9–20 mm		
Caryophyllaceae	*Vaccaria pyramidata* Medik.	Cowherb (Kermanshah)	Shoot	Methanolic extract	250 ppm-MFC	*A. candida*	Omranpour et al. (2011)
Ephedraceae	*Ephedra major* Host.	Ephedra	Roots	Methanolic extract	>1,000 µg/ml-MIC	*A. parasiticus*	Bagheri-Gavkosh et al. (2009)
Fabaceae	*Glycyrrhiza glabra*	Licorice	Leaves	80 % ethanolic extract	25.0–83.3 mg/ml-MICs	*A. flavus* *A. fumigatus* *A. niger*	Rashidi et al. (2011)
	Dalbergia sissoo	Amerimnon sissoo (Khuzestan)	Aerial parts	80% ethanolic extract	500–2,000 µg/ml-MICs	*A. niger*	Arabi and Sardari (2010)

(continued)

Table 2.1 (continued)

Family	Species	Common name (place of collection)	Part used	Effective component (s)	Inhibitory concentration	Affected fungi	Reference
						C. albicans	
						A. fumigatus	
	Lathyrus pratensis	Meadow pea (Mazandaran)					
	Hippocrepis unisiliquosa L.	Budellina (Khuzestan)					
	Oreophysa microphylla Bunge ex Boiss.	-(Tehran)					
	Onobrychis altissima Grossh.	Chickwood (Ardebil)					
	Argyrolobium roseum (Cambess.) Jaub. & Spach	-(Hormozgan)					
	Hymenocarpus circinnatus (L.) Savi.	Disk trefoil (Lorestan)					
	Astragalus stepporum	Milk-vetch (Khuzestan)					
	Ammodendron persicum	-(Khorasan)					
	Sophora alopecuroides L.	Lighiyellow sophora (Isfahan)					
	Taverniera cuneifolia (Roth)	Taverniera (Kerman)					
	Ebenus stellata Boiss.	Ebenus (Fars)					
Fagaceae	*Ziziphus spina-christi* (L.) Willd.	Nubk tree (Ilam)	Fruits	Aqueous extract	10–55 μg/disk	*C. albicans*	Ghasemi-Pirbalouti et al. (2009)
					Inhibition zone of 18–34 mm		
Geraniaceae	*Pelargonium graveolens*	Geranium	Aerial parts	Essential oil (citronelol and geraniol as the main constituents; 28.2 and 22.1 %)	30 μl/disk	*M. furfur*	Naeini et al. (2011)
						M. globosa	
						M. obtusa	
				Essential oil	250 μg/ml	*C. albicans*	Naeini et al. (2009)

(continued)

Table 2.1 (continued)

Family	Species	Common name (place of collection)	Part used	Effective component (s)	Inhibitory concentration	Affected fungi	Reference
Hypericaceae	*Hypericum perforatum* L.	Amber (Kermanshah)	Shoot	Methanolic extract	100 ppm-MFC	*A. candida*	Omranpour et al. (2011)
Illiciaceae	*Illicium verum* Hook. f.	Star anise	Fruits	Methanolic extract	4–64 mg/ml	*A. niger* *C. albicans* *M. canis* *T. mentagrophytes* *E. floccosum*	Yazdani et al. (2009)
Lamiaceae (Labiateae)	*Hymenocrater calycinus* (Boiss.) Benth.	Oraman's tulip (Firuzkuh)	Aerial parts	Rosmarinic acid (from ethyl acetate and methanolic extracts)	250 μg/ml	*C. albicans*	Gohari et al. (2009)
					1,000 μg/ml-MICs	*A. niger*	
	Origanum vulgare	Oregano (Ghom-Markazi)	Aerial parts	Essential oil (linalool, and thymol as the main constituents; 42.0 and 25.1 %)	0.3–1,100 μg/ml-MICs	*C. glabrata*	Khosravi et al. (2011)
					2.2–2,200 μg/ml-MFCs		
	Salvia officinalis	Common sage	Leaves	80% ethanolic extract	16.67–133.34 mg/ml-MICs	*A. flavus*	Rashidi et al. (2011)
	Lavandula officinalis	Lavender				*A. fumigatus* *A. niger*	
	Zataria multiflora Boiss. Sattar	Zattar Avishan	Aerial parts	Essential oil (thymol as the main constituent; 50–70 %)	0.25–2 mg/ml	*C. albicans*	Saei-Dehkordi et al. (2010)
					0.062–0.5 mg/ml-MICs	*C. tropicalis*	
					≥400 ppm-MFC	*A. flavus*	Gandomi et al. (2009)
				Essential oil (carvacrol as the main constituent; ~60 %)	30 μl/disk	*M. furfur*	Naeini et al. (2011)
						M. globosa *M. obtusa*	
				Essential oil	66–612 μg/ml-MICs	*F. solani* *F. oxysporum* *F. verticillioides*	Naeini et al. (2010)

(continued)

Table 2.1 (continued)

Family	Species	Common name (place of collection)	Part used	Effective component (s)	Inhibitory concentration	Affected fungi	Reference
						F. poae	
						F. equiseti	
				Essential oil	150 μg/ml-MIC	*C. albicans*	Naeini et al. (2009)
				Essential oil	8–256 μg/ml	*A. niger*	Ghasemi-Pirbalouti et al. (2011)
						A. fumigatus	
					128 μg/ml; MFC	*A. flavus*	
						A. parasiticus	
	Satureja Khuzistanica Jamzad.	Marzeh (Khoramabad-Lorestan)	Aerial parts	Methanolic extract	2–8 mg/ml	*A. niger*	Amanlou et al. (2004)
					1–4 mg/ml-MICs	*C. albicans*	
		Marzeh (Dezful-Khuzestan)		80 % ethanolic extract	625-2,500 μg/ml-MICs	*A. flavus*	Sadeghi-Nejad et al. (2010)
						A. niger	
						Penicillium sp.	
						Fusarium sp.	
						Alternaria sp.	
						Rhizopus sp.	
						Mucor sp.	
	Ziziphora clinopodioides Lam.	Kakuti-e-Kuhi	Aerial parts	Essential oil	560 μg/ml-MIC	*C. albicans*	Naeini et al. (2009)
	Satureja hortensis	Summer savory Koc Out	Leaves	Thymol	0.86 μM	*A. parasiticus*	Razzaghi-Abyaneh et al. (2008)
				Carvacrol	0.79 μM-IC_{50}		
			Aerial parts	Essential oil	1,100 μg/ml-MIC	*C. albicans*	Naeini et al. (2009)
				Essential oil (thymol, *p*-cymene, γ-terpinene and carvacrol as the main constituents; 28.2, 19.6, 16.0 and 11.0 %)	0.06–1 μg/ml-MICs	*C. albicans*	Mahboubi and Kazempour (2011)
						C. glabrata	
						A. niger	

(continued)

Table 2.1 (continued)

Family	Species	Common name (place of collection)	Part used	Effective component (s)	Inhibitory concentration	Affected fungi	Reference
						A. flavus	
						A. parasiticus	
	Mentha spicata	Spearmint (Yazd)	Aerial parts	Essential oil	1–5 µl disks on PDA for 4 days at 25 C	*F. oxysporum* f. sp. *radicis-cucumerinum*	Nosrati et al. (2011)
				Essential oil	2,300 µg/ml	*C. albicans*	Naeini et al. (2009)
			Leaves	Essential oil (piperitenone oxide and *cis*-carveol as the main constituents; 34.7 and 21.7 %)	35 µg/ml-IC_{50}	*A. parasiticus*	Alinezhad et al. (2011)
	Mentha piperita	Spearmint (Damavand-Tehran)	Leaves	Essential oil (α-terpinene, pipertitinone oxide, *trans*-carveol and isomanthone as the main constituents; 19.7, 19.3, 14.5 and 10.3 %)	1 µl/ml-MIC	*C. albicans*	Yadegarinia et al. (2006)
					≥2 µl/ml-MLC		
	Stachys inflata Benth.	–(Kashan-Isfahan)	Aerial parts	Essential oil	No effect	*C. albicans*	Ebrahimabadi et al. (2010a)
				– Linalool (28.55 % of the oil)	125-500 µg/ml	*A. niger*	
				– α-terpineol (9.45 % of the oil)	250–500 µg/ml		
				Methanolic extract	250 µg/ml only for *C. albicans*-MICs		
	Hymenocrater longiflorus Benth.	Oraman's tulip (Kermanshah)	Aerial parts	Essential oil (Δ-cadinol and α-pinene as the main constituents; 18.49 and 10.16 %) Methanolic extract	240–480 µg/ml-MICs	*C. albicans*	Ahmadi et al. (2010)

(continued)

Table 2.1 (continued)

Family	Species	Common name (place of collection)	Part used	Effective component (s)	Inhibitory concentration	Affected fungi	Reference
					>10 μg/ml-MICs	*A. niger*	
	Salvia eremophila Boiss.	Sage (Kashan-Isfahan)	Aerial parts	Essential oil (borneol, α-pinene and bornyl acetate as the main constituents; 21.83, 18.80 and 18.68 %)	250 μg/ml	*C. albicans*	Ebrahimabadi et al. (2010b)
					>500 μg/ml-MICs	*A. niger*	
				Methanolic extract	No effect		
	Rosmarinus officinalis Linn.	Rosemary	Aerial parts	Essential oil	2,300 μg/ml-MIC	*C. albicans*	Naeini et al. (2009)
	Thymus eriocalyx	Thyme	Aerial parts	Essential oil (thymol as the main constituent; 64.3 %)		*A. parasiticus*	Rasooli and Razzaghi-Abyaneh (2004)
	Thymus x-prolock			Essential oil (ß-phellandrene as the main constituent; 39.4 %)			
	Thymus vulgaris	Thyme	Leaves	Essential oil (thymol as the main constituent; 70.99 %)	1,000 μg/ml-MFC	*A. parasiticus*	Razzaghi-Abyaneh et al. (2009)
	Thymus daenensis Celak.	Thyme (Chaharmahal va Bakhtiari)	Aerial parts	Essential oil	10–55 μl/disk	*C. albicans*	Ghasemi-Pirbalouti et al. (2009)
					Inhibition zone of 20–23 mm		
	Thymbra spicata L.	Thyme (Ilam)					
	Satureja bachtiarica Bunge.	Savory (Chaharmahal va Bakhtiari)					
Liliaceae	*Allium heamanthoides*	Musir (Kermanshah)	Corm	Methanolic extract	250 ppm-MFC	*A. candida*	Omranpour et al. (2011)
Lythraceae	*Lawsonia inermis* L.	Henna	Leaves	Extracts (aqueous, chloroformic, methanolic)	0.25–4 %; v/v	*Malassezia* sp.	Berenji et al. (2010)
Malvaceae	*Althaea officinalis*	Marshmallow	Leaves	80 % ethanolic extract	50.0–83.34 mg/ml-MICs	*A. flavus*	Rashidi et al. (2011)

(continued)

Table 2.1 (continued)

Family	Species	Common name (place of collection)	Part used	Effective component (s)	Inhibitory concentration	Affected fungi	Reference
						A. fumigatus	
						A. niger	
Meliaceae	*Azadirachta indica* A. Juss	Neem	Leaves seeds	Aqueous extracts	>90 % inhibition at 50 % v/v concentrations	*A. parasiticus*	Allameh et al. (2001)
		Margosa (Bandarabbas)					Razzaghi-Abyaneh et al. (2005)
							Ghorbanian et al. (2007)
			Seeds	Essential oil	66.4 % inhibition at 1,000 µg/ml	*A. parasiticus*	Razzaghi-Abyaneh et al. (2009)
Myrtaceae	*Myrtus commonis* Linn.	Myrtle	Leaves	Essential oil (1,8-cineole, α-pinene and linalool as the main constituents; 36.1, 22.5 and 8.4 %)	8–16 µg/ml-MICs	*C. albicans*	Mahboubi and Bidgoli (2010)
						A. niger	
						A. flavus	
						A. parasiticus	
				Essential oil (α-pinene, limonene, 1,8-cineole and linalool as the main constituents; 29.1, 21.5, 17.9 and 10.4 %)	2 µl/ml		
-MIC	*C. albicans*	Yadegarinia et al. (2006)					
					4–8 µl/ml-MLC		
	Syzygium aromaticum (Eugenia caryophyllata)	Clove (Kermanshah)	Shoot	Methanolic extract	100 ppm-MFC	*A. candida*	Omranpour et al. (2011)
Nitrariaceae	*Peganum harmala*	African rue (Kashan)	Seeds	Aqueous extract	1.0 %, v/v in PDA plates	*Alternaria* sp.	Sarpeleh et al. (2009)

(continued)

Table 2.1 (continued)

Family	Species	Common name (place of collection)	Part used	Effective component (s)	Inhibitory concentration	Affected fungi	Reference
						B. cinerea *C. cucumerinum* *C. cassilicola* *F. oxysporum* *M. phaseolina* *P. drechsleri* *T. harzianum* *Ulocladium* sp. *V. dahliae*	
Papaveraceae –active against MC, MG, TM and EF	*Glaucium oxylobum* Boiss. et Buhse	Horned poppy Morteza-Semnani et al. (2003)	Aerial parts	Methanolic extract	10 mg/ml		
		Lotus sweetjuice (Roodbar-Guilan)				*M. gypseum* (MG)	
						T. mentagrophytes (TM)	
				300 μg/ml of isolated alkaloids:			
						E. floccosum (EF)	
				– Dicentrine	– Active against MC, MG, TM and EF		
				– Glaucine	– Active against MC, MG and TM		
				– Protopine	– Active against MC and TM		
				– α-allocryptopine	– Active against MG and EF		
				– *O*-methylflavinantine	– No antifungal activity		
Poaceae	*Avena sativa* L.	Common oat	Roots	Methanolic extract	5 mg/disk Inhibition zone of 6.1–9.6 mm	*R. solani*	Bahraminejad et al. (2011)

(continued)

Table 2.1 (continued)

Family	Species	Common name (place of collection)	Part used	Effective component (s)	Inhibitory concentration	Affected fungi	Reference
						F. oxysporum	
Primulaceae	*Anagallis arvensis* L.	Red chickwood	Whole plant				
						C. sativus	
		Red chickwood (Kermanshah)	Shoot	Methanolic extract	50 ppm-MFC	*A. candida*	Omranpour et al. (2011)
Ranunculaceae	*Nigella sativa*	Black cumin Siyahdaneh	Aerial parts	Essential oil	2,300 μg/ml-MIC	*C. albicans*	Naeini et al. (2009)
Rhamnaceae	*Quercus brantii* Lindl.	Oak manna tree (Ilam)	Fruits	Aqueous extract	10–55 μg/disk inhibition zone of 6–7 mm	*C. albicans*	Ghasemi-Pirbalouti et al. (2009)
Rosaceae	*Mespilus germanica*	Common medlar (Kermanshah)	Leaves	Methanolic extract	50 ppm-MFC	*A. candida*	Omranpour et al. (2011)
Rutaceae	*Citrus aurantifolia* Swingle	Aour lime	Leaves	Essential oil (limonene as the main constituent; 85.5 %)	1,000 μg/ml-IC_{50}	*A. parasiticus*	Razzaghi-Abyaneh et al. (2009)
Scrophulariaceae	*Scrophularia striata* Boiss.	Figwort	Leaves	Aqueous extract	10–55 μg/disk inhibition zone of 22–35 mm	*C. albicans*	Ghasemi-Pirbalouti et al. (2009)
	Verbascum nigrum L.	Dark Mullein	Shoot	Methanolic extract	100 ppm-MFC	*A. candida*	Omranpour et al. (2011)
Zingiberaceae	*Zingiber officinale* Rosc.	Ginger (Kermanshah)	Rhizome	Methanolic extract	100 ppm-MFC	*A. candida*	Omranpour et al. (2011)
Zygophyllaceae	*Tribulus terrestris* L.	Puncture vine	Shoot	Aqueous and methanolic extracts	5 mg/disk	*R. solani*	Bahraminejad et al. (2011)
						F. oxysporum	
					Inhibition zone of 6.6–17.6 mm		
						C. sativus	
			Roots				
		Caltrop					
		Yellow vine					
Plant resin-like exudates	Iranian Propolis	Balsam (Tehran-Khojir)	Exudates	Ethanolic extract (70 % in water)	250 μg/ml	*C. albicans*	Mohammadzadeh et al. (2007)
					500 μg/ml	*A. niger*	

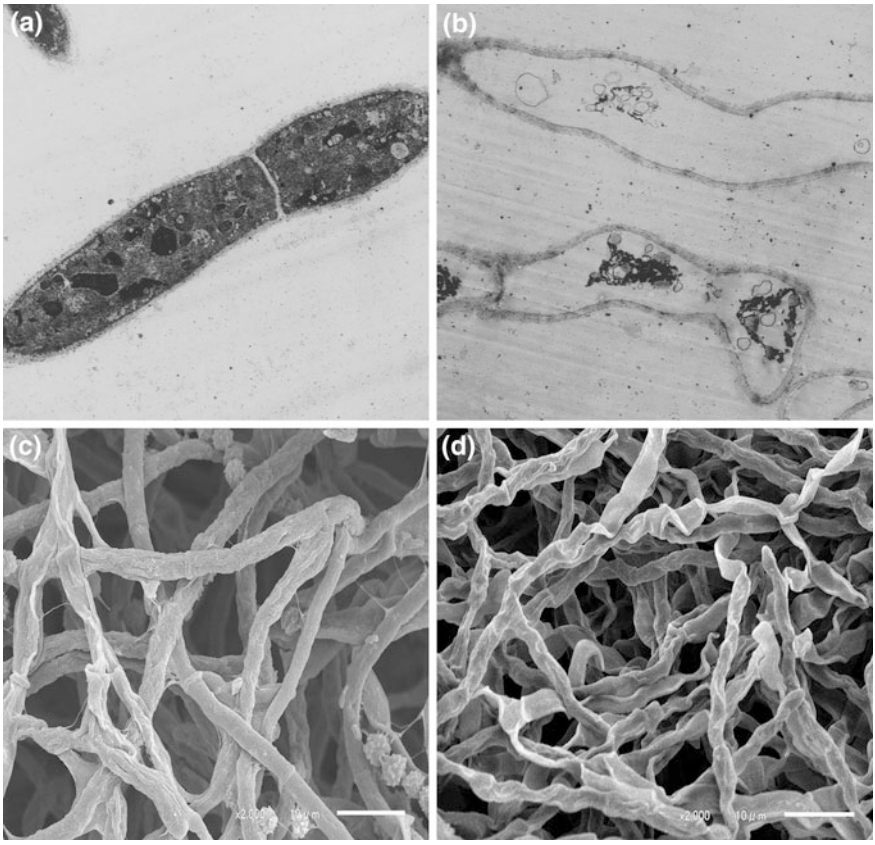
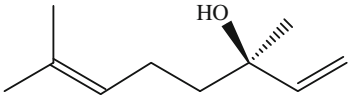


Fig. 2.1 Electron microscopic study of *A. niger* grown on PDB with or without *M. chamomilla* flower essential oil (EO; 1,000 μg/ml) after 96-h incubation at 28 °C. Transmission electron micrographs (*TEM*) of control (untreated) fungal mycelia (**a**) show uniform hypha with normal septum, walls, and organelles, while TEM of EO-treated sample (**b**) indicates deformation of hyphae and massive destruction of cellular organelles. Scanning electron micrographs (*SEM*) reveal normal conidia and hyphae and conidiophores with uniform tubular shape in all parts for control sample (**c**), while it shows massive collapse and folding of whole hyphae and lack of conidiation for EO-treated sample (**d**)

Stachys inflata Benth. (Persian names: poulk, gole arghavan) is one of the endemic species of Iran with routine application as herbal tea in the treatment for various infections and inflammatory disorders (Zargari 1992).

Methanolic extract of plant aerial parts was shown to effectively inhibit the growth of *Candida albicans*, while the plant essential oil did not affect fungal growth even at a concentration of 1,000 μg/ml (Ebrahimabadi et al. 2010a). The main plant constituents, that is, linalool **1** ($C_{10}H_{18}O$, 154.24 g/mol) and α-terpineol **2** ($C_{10}H_{18}O$, 154.24 g/mol), were shown to be responsible for antifungal properties with minimal inhibitory concentrations (MICs) between 125 and 500 μg/ml for *C.*

albicans and *A. niger*.



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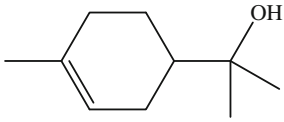
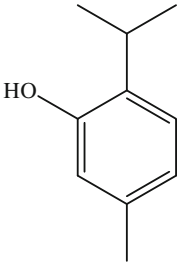


2

2.2.3 Satureja hortensis

The genus *Satureja* (Lamiaceae) comprises about 30 species called savories (Marzeh in Persian) with 12 species found in Iran of which three species, that is, *S. hortensis* ("summer savory" or "Koc Out"), *S. khuzistanica*, and *S. bachtiarica*, are the most important ones. Besides the routine use in food industry as aromatic and flavoring agents, they have received major consideration due to their anti-inflammatory, antioxidant, antibacterial, and antifungal properties (Sharafzadeh and Alizadeh 2012).

Methanolic extracts of aerial parts of *S. khuzistanica* showed strong inhibitory activity toward the growth of *C. albicans* and *A. niger* with MICs of 1–8 mg/ml (Amanlou et al. 2004). Essential oil of aerial parts of *S. bachtiarica* is found to be effectively inhibiting cell growth of *C. albicans* in concentrations of 10–55 μg/disk in disk diffusion assay (Ghasemi-Pirbalouti et al. 2009). Essential oil of *S. hortensis* leaves inhibited mycelia growth of aflatoxigenic *A. parasiticus* dose-dependently (Razzaghi-Abyaneh et al. 2008). The leaves were reported to contain thymol **3** ($C_{10}H_{14}O$, 150.21 g/mol) and carvacrol **4** ($C_{10}H_{14}O$, 150.21 g/mol) as effective antifungal components with IC_{50} values of 0.86 and 0.79 μM, respectively.



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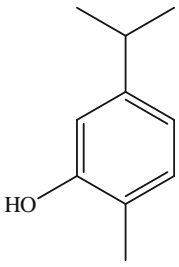

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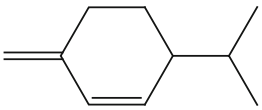
2.2.4 *Thymus vulgaris*

The genus *Thymus* (Lamiaceae) comprises about 350 species of aromatic perennial plants which are native to temperate regions of Europe, North Africa, and Asia. The plant leaves are fragrant and best known for antispasmolytic, antibacterial, antifungal, antiseptic, carminative, anthelmintic, and antitussive properties. In this genus, four species growing in Iran are identified to have growth inhibitory activities against a wide array of fungal pathogens of which *T. vulgaris* (green thyme) is the most important species distributed in all the countries.

Essential oil of leaves of *T. vulgaris* was found to strongly inhibit mycelia growth of an aflatoxigenic *Aspergillus parasiticus* with minimal fungicidal concentration (MFC) value of 1,000 μg/ml (Razzaghi-Abyaneh et al. 2009). Essential oils of *T. x-prolock* and *T. eriocalyx* aerial parts inhibited the growth of *A. parasiticus* dose-dependently (Rasooli and Razzaghi-Abyaneh 2004). The aerial parts were reported to contain *β*-phellandrene **5** ($C_{10}H_{16}$, 136.23 g/mol) and thymol **3** as the main constituents which are reported to have antifungal properties. Growth inhibition of the yeast pathogen, *C. albicans*, has been reported for the fungus exposed to 10–55 μl/disk of essential oil of *T. daenensis* aerial parts (Ghasemi-Pirbalouti et al. 2009).



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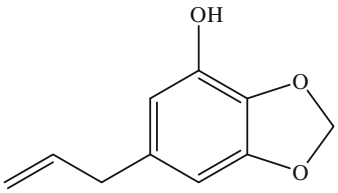
2.2.5 *Heracleum persicum*

The genus *Heracleum* (Golpar in Persian) with 120 species in the world is widely distributed in Asia. Within eight species exist in the flora of Iran, four including *H. persicum*, *H. rechingeri*, *H. pastinacifolium*, and *H. transcaucasicum* are reported to have antifungal properties. *H. persicum* as the most important species is indigenous to the moist valleys of the Elburz Mountains and is related to *H. pubescens* of a wider range. The fruits and leaves of this genus are used as odorant, antimicrobial, antiseptic, carminative, digestive, and analgesic in the Iranian folk medicine.

Essential oil of aerial parts of *H. persicum* was reported to inhibit the growth of *A. niger*, *C. albicans*, and some *Fusarium* species with MIC values of 70–4,500 and 1100 μg/ml for two latter fungi (Firuzi et al. 2010; Naeini et al. 2010). Ethyl acetate extract of plant seed showed strong inhibitory activity against *A. parasiticus* by an IC_{50} value of 370 μg/ml (Alinezhad et al. 2011). In a disk diffusion assay, essential oils of aerial parts of *H. rechingeri*, *H. pastinacifolium*, and *H. transcaucasicum* were reportedly inhibiting the growth of *A. niger* and *C. albicans*

(Firuzi et al. 2010). The main oil constituents of these species and *H. persicum* reported to have antifungal properties were identified by the authors as myristicin **6** ($C_{11}H_{12}O_3$, 192.21 g/mol), elemicin **7** ($C_{12}H_{16}O_3$, 208.25 g/mol), hexyl butanoate **8** ($C_{10}H_{20}O_2$, 172.26 g/mol), and *trans*-anethole **9** ($C_{10}H_{12}O$, 148.20 g/mol), respectively.



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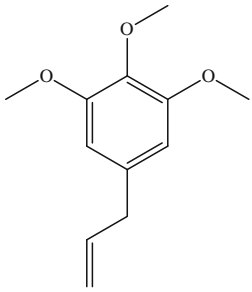

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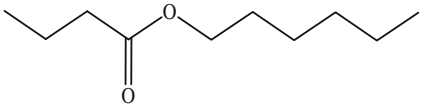

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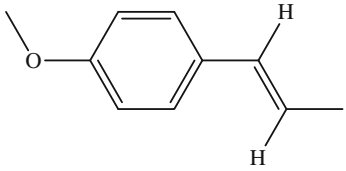

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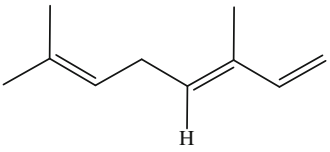
2.2.6 *Artemisia dracunculus*

The genus *Artemisia* (Asteraceae) comprises 200–400 species of which many of them possess different biological activities owing to have a wide range of secondary metabolites like flavonoids, phenylpropanoids, coumarins, terpenoids, and sesquiterpene lactones. *A. dracunculus* L. (Azerbaijan tragon) is one of the most important species native to northwest of Iran which has a long history of use in culinary traditions. It is cultivated 1,340 m above sea level in Urmia, and the leaves are well known for antimicrobial, laxative, carminative, and antispasmodic properties. Usually, an infusion made from a teaspoon of its twigs is consumed an hour before meals.

Essential oil of *A. dracunculus* leaves reportedly showed antifungal activity against aflatoxigenic *A. parasiticus* with a maximum of 70 % fungal growth inhibition at 1,000 μg/ml (Razzaghi-Abyaneh et al. 2009). The main oil constituents, that is, *trans*-ocimene **10** ($C_{10}H_{16}$, 136.23 g/mol), *trans*-anethole **9**, and terpinolene **11** ($C_{10}H_{16}$, 136.23 g/mol), were found to be responsible for plant

biological activities. Essential oils of aerial parts of three other native species including *A. scoparia*, *A. sieberi*, and *A. kermanensis* inhibited the growth of pathogenic yeasts, *C. albicans* and *C. glabrata*, by microbroth and disk diffusion methods (Ramezani et al. 2004; Naeini et al. 2009; Khosravi et al. 2011). Camphor **12** ($C_{10}H_{16}O$, 152.23 g/mol) and *β*-thujone **13** ($C_{10}H_{16}O$, 152.23 g/mol), the main oil constituents of *A. sieberi*, are well known to have antifungal properties.



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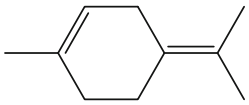

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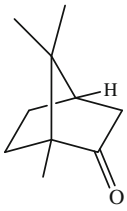

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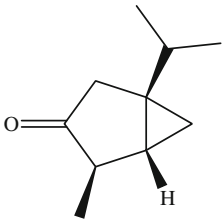

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2.2.7 *Zataria multiflora*

Zataria multiflora Boiss. (Zattar; Lamiaceae) is a thyme-like plant which grows wild in central and southern parts of Iran. Globally, it is cultivated only in warm climates of Iran, Afghanistan, and Pakistan. The plant is well known as a remedy due to its odorant, food preservative, antispasmodic, antiseptic, analgesic, carminative, anesthetic, antinociceptive and antibacterial and antifungal properties (Zargari 1992). The plant is a rich source of bioactive metabolites suitable for producing medicines. Today, efforts are made to develop it as expectorant, cough suppressant, vaginal douche and pain-relieving creams and antimicrobial and antifungal mouth wash in Iran.

Essential oil of plant aerial parts was shown to effectively inhibit the growth of a wide array of pathogenic yeasts and molds (Gandomi et al 2009; Naeini et al. 2009, 2010, 2011; Saei-Dehkordi et al. 2010; Ghasemi-Pirbalouti et al. 2011). The growth of *C. albicans* and *C. glabrata* was significantly inhibited by the plant oil with MICs of 62–2,000 μg/ml for different fungal strains (Naeini et al. 2009; Saei-Dehkordi et al. 2010). In a disk diffusion assay, the plant oil exhibited strong antifungal activity against main pathogenic *Malassezia* species including

M. furfur, *M. globosa*, and *M. obtusa* in a concentration of 30 μg/disk (Naeini et al. 2011). The growth of various species of pathogenic molds from the genera *Aspergillus* (*A. niger*, *A. flavus*, *A. fumigatus*, and *A. parasiticus*) and *Fusarium* (*F. equiceti*, *F. oxysporum*, *F. poae*, *F. solani*, and *F. verticillioides*) was strongly inhibited by the oil with MICs of 8–256 μg/ml and 66–62 μg/ml, respectively (Gandomi et al. 2009; Naeini et al. 2010; Ghasemi-Pirbalouti et al. 2011).

Thymol **3** and carvacrol **4** identified as main oil constituents of *Z. multiflora* aerial parts may account for the plant antifungal properties.

2.2.8 *Achillea millefolium* subsp. *elborsensis*

A. millefolium (Asteraceae) is a flowering plant grown in any well-drained soil in full sun. Besides the diaphoretic and astringent properties, the plant is well known as a "healing herb" used topically for treatment for wounds, cuts, and abrasions. It has been reported to contain a wide array of bioactive metabolites including asparagine, bitters, coumarins, flavonoids, isovaleric and salicylic acids, and tannins. Among more than 10 subspecies identified, *A. millefolium* subsp. *elborsensis* is native to Alborz Mountains extending about 650 km from west to east along the border of Iran, at the southern shore of the Caspian Sea (Kamrani et al. 2011).

In the only documented work on antifungal activity of this plant variety, a powerful growth inhibitory activity was reported against aflatoxigenic *A. parasiticus* exposed to the flowers' essential oil with an IC_{50} value of 580 μg/ml (Alinezhad et al. 2011). Chamazulene **14** ($C_{14}H_{16}$, 184.27 g/mol) identified as the main constituent of plant oil by the authors may account for antifungal properties due to its proven antimicrobial activities against a wide array of microorganisms.

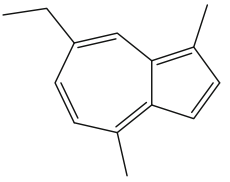
2.2.9 *Hymenocrater calycinus*

The genus *Hymenocrater* Fisch.et Mey., (Lamiaceae) named Gol-e-Arvaneh in Persian, comprises about eleven species, nine of them reported in Iran of which four species including *H. longiflorus*, *H. calycinus*, *H. yazdianus*, and *H. incanus* are endemic. *H. calycinus* is growing wildly in the northeast of Iran and some parts of Turkmania as a native plant. Another important species, that is, *H. longiflorus*, with strong characteristic aroma is only distributed in Oramanat region, Kermanshah Province, where it is commonly known as "Oraman's tulip" used as a house freshener and an antimosquito agent.

Among four compounds isolated from ethyl acetate and methanolic extracts of *H. calycinus* aerial parts which identified as *β*-sitosterol, ursolic acid, rosmarinic acid, and quercetin 3-O-rutinoside by NMR and MS spectral data, only rosmarinic acid **15** ($C_{18}H_{16}O_8$, 360.31 g/mol) showed antifungal activity against *C. albicans*

and *A. niger* in broth dilution assay (Gohari et al. 2009). MIC values of the compound for these fungi were reported as 250 and 1,000 μg/ml, respectively.

Essential oil of aerial parts of *H. longiflorus* exhibited strong inhibitory activity against *C. albicans* and *A. niger* with MICs of 240–480 μg/ml (Ahmadi et al. 2010). Δ-cadinol **16** ($C_{15}H_{26}O$, 222.36 g/mol) and α-pinene **17** ($C_{10}H_{16}$, 136.23 g/mol) were identified as main constituents account for 18.49 and 10.16 % of plant oil, respectively.



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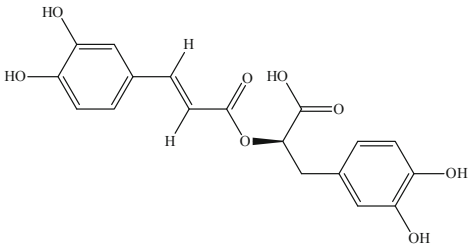

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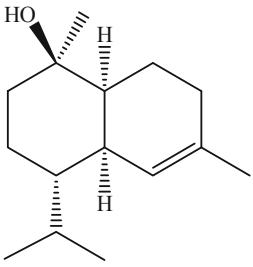

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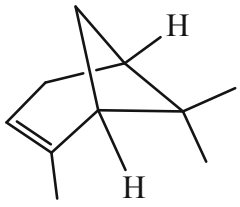

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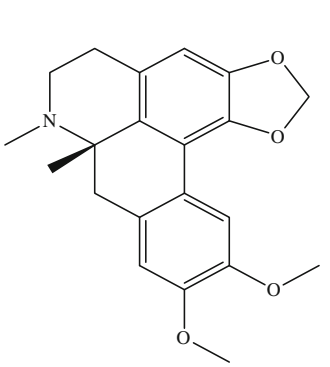
2.2.10 Glaucium oxylobum

The genus *Glaucium* (Papaveraceae) named Lal-e-Koohi in Persian contains about 25 species native to Europe, North Africa, and Southwest and Central Asia of which 19 species are found in Iran as one of the most plant habitats in the world. *Glaucium* species are known in Iranian traditional medicine for their laxative, hypnotic, antidiabetic, and antimicrobial properties.

The methanolic extract and total alkaloids of the aerial parts of *G. oxylobum* showed strong inhibitory activity against *Microsporum gypseum, Microsporum canis, Trichophyton mentagrophytes*, and *Epidermophyton floccosum* as the main causative fungal species of zoonotic dermatophytosis (Morteza-Semnania et al. 2003). Four alkaloids, that is, dicentrine **18** ($C_{20}H_{21}NO_4$, 339.38 g/mol), glaucine **19** ($C_{21}H_{25}NO_4$, 355.42 g/mol), protopine **20** ($C_{20}H_{19}NO_5$, 353.36 g/mol),

and α-allocryptopine **21** ($C_{21}H_{23}NO_5$, 369.41 g/mol), were identified by the authors as the bioactive compounds responsible for antifungal activity of this plant. Methanolic extract and isolated plant alkaloids were effective against tested dermatophytes at concentrations of 1,000 and 300 μg/ml, respectively. The fifth alkaloid, *O*-methylflavinantine, did not show any inhibitory effect on tested fungi. Neither isolated alkaloids nor plant methanolic extract was able to inhibit the growth of *C. albicans*, *A. niger*, and *Penicillium* sp.



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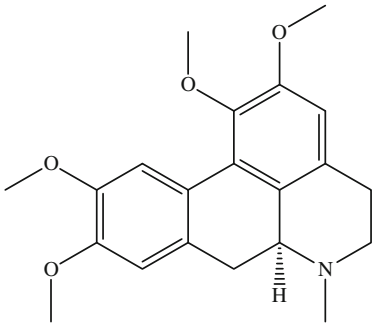

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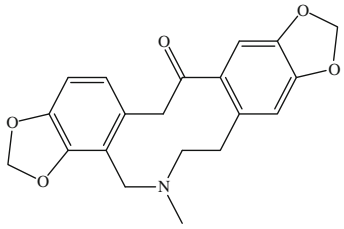

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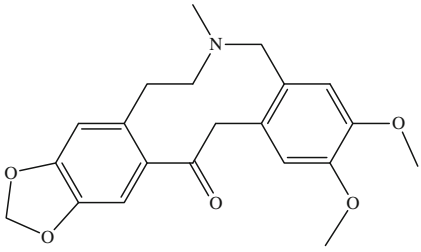

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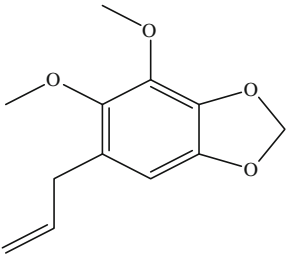
2.2.11 Diplotaenia damavandica

The genus *Diplotaenia* (Apiaceae) was described by Boissier (1844) as a monotypic genus based on *D. cachrydifolia* Boiss. from Iran. *D. damavandica* (kozal in Persian) is a perennial wild herb exclusively found in central Alborz Mountains around Tar Lake, Damavand, Iran. The extracts obtained from the aerial parts of the plant have been reported to have antifungal activity against the genera *Aspergillus*, *Candida*, and *Cryptococcus* with furanocoumarins as the effective constituents (Sardari et al. 2000).

Essential oil of plant leaves reportedly inhibited the growth of *C. albicans*, *Saccharomyces cereviciae*, and *A. niger* with MICs of 3.6–7.2 mg/ml (Eftekhar et al. 2005).The leaves constituents terpinolene **11**, dillapiole **22** ($C_{12}H_{14}O_4$, 222.23 g/mol), γ-terpinene **23** ($C_{10}H_{16}$, 136.23 g/mol), and *p*-cymene **24** ($C_{10}H_{14}$, 134.21 g/mol) were shown to have strong antifungal activities against tested fungi with MICs between 6.6 and 110.1 mM.



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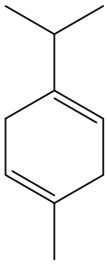

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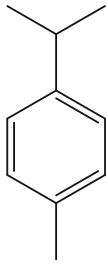

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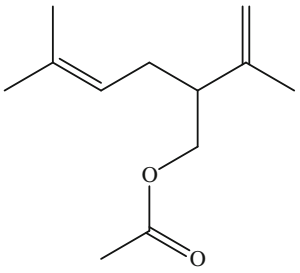
2.2.12 Semenovia tragioides

The genus *Semenovia* (Apiaceae) consists of about 300 genera and 3,000 species of aromatic permanent plants growing mostly in the mountain regions of Iran and Turkey, and Mediterranean region (Ghahreman 1995). The plant is mainly used as edible vegetable and as sources of volatile oils and drugs. Among 20 *Semenovia* species growing in Asia, eleven are found in Iran and five are endemic. *Semenovia tragioides* is one of the most important endemic species of Iran that its fragrant leaves and fruits are frequently used as carminative and flavoring agent in southwest Iran.

Essential oils of aerial parts of *S. tragioides* exhibited strong antifungal activity against pathogenic yeast *C. albicans* with MIC of 5 mg/ml, while the plant oil did not show any obvious effect on the growth of mycelia fungus *A. niger* (Bamoniri et al. 2010). The main compounds identified by the authors in plant oil including lavandulyl acetate **25** ($C_{12}H_{20}O_2$, 196.28 g/mol), geranyl acetate **26** ($C_{12}H_{20}O_2$, 196.28 g/mol), *trans*-ocimene **10**, *p*-cymene **24**, and γ-terpinene **23** may account for its antifungal properties.



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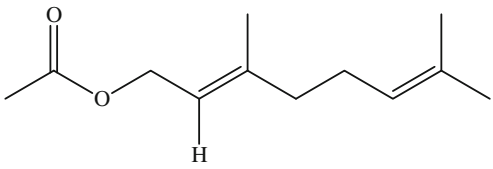

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2.3 Concluding Remarks and Future Perspectives

Dramatic increase in emerging fungal diseases, restricted availability of antifungal drugs suitable for treating systemic fungal infections, and appearance of multi-drug-resistant fungi as major global health problem in the twenty-first century urge the health organizations and pharmaceutical industries to return back to biodiversity for more effective, safer, and broad spectrum antifungals that battle against these life-threatening microbial infections. Among existing biodiversity, medicinal plants are still in the first line of investigation owing to have a large number of bioactive metabolites with huge structural diversities. In this chapter, we carried out a comprehensive literature review with the aim to introduce antifungal activities of medicinal plants from flora of Iran. We characterized 83 native medicinal plants with proven benefits against a wide array of fungal pathogens. Actually, majority of enlisted plants were shown to contain different numbers of bioactive constituents with strong antifungal activities when isolated from same or other plants in bioassays. The α-bisabolol from *M. chamomilla* is a very good example of such compounds which has a potential for being a unique antifungal drug candidate. We believe these comprehensive data could help the researchers in further phytochemical characterization of medicinal plants, which finally will lead to development of more effective drugs and candidates suitable for treatment for life-threatening fungal infections. Development of bioinformatic and computational chemistry approaches to routine screening for antifungal drug discovery from medicinal plants will accelerate such process in a best way.

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References

Abdallah EM (2011) Plants: an alternative source for antimicrobials. J Appl Pharmaceut Sci 1:16–20

Ahmadi F, Sadeghi S, Modarresi M, Abiri R, Mikaeli A (2010) Chemical composition, in vitro anti-microbial, antifungal and antioxidant activities of the essential oil and methanolic extract of *Hymenocrater longiflorus* Benth of Iran. Food Chem Toxicol 48:1137–1144

Alinezhad S, Kamalzadeh A, Shams-Ghahfarokhi M, Rezaee MB, Jaimand K, Kawachi M, Zamani Z, Tolouei R, Razzaghi-Abyaneh M (2011) Search for novel antifungals from 49 indigenous medicinal plants: *Foeniculum vulgare* and *Platycladus orientalis* as strong inhibitors of aflatoxin production by *Aspergillus parasiticus*. Ann Microbiol 61:673–681

Allameh A, Razzaghi-Abyaneh M, Shams M, Rezaee MB, Jaimand K (2001) Effects of neem leaf extract on production of aflatoxins and activities of fatty acid synthetase, isocitrate dehydrogenase and glutathione S-transferase in *Aspergillus parasiticus*. Mycopathologia 154:79–84

Amanlou M, Fazeli MR, Arvin A, Amin HG, Farsam H (2004) Antimicrobial activity of crude methanolic extract of *Satureja khuzistanica*. Fitoterapia 75:768–770

Arabi Z, Sardari S (2010) An investigation into the antifungal property of Fabaceae using bioinformatics tools. Avic J Med Biotechnol 2:93–99

Bagheri-Gavkosh S, Bigdeli M, Shams-Ghahfarokhi M, Razzaghi-Abyaneh M (2009) Inhibitory effects of *Ephedra major* Host on *Aspergillus parasiticus* growth and aflatoxin production. Mycopathologia 168:249–255

Bahraminejad S, Abbasi S, Fazlali M (2011) *In vitro* antifungal activity of 63 Iranian plant species against three different plant pathogenic fungi. Afr J Biotechnol 10:16193–16201

Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008) Biological effects of essential oils: a review. Food Chem Toxicol 46:446–475

Balunas MJ, Kinghorn AD (2005) Drug discovery from medicinal plants. Life Sci 78:431–441

Bamoniri A, Ebrahimabadi AH, Mazoochi A, Behpour M, Jookar-Kashi F, Batooli H (2010) Antioxidant and antimicrobial activity evaluation and essential oil analysis of *Semenovia tragioides* Bioss. from Iran. Food Chem 122:553–558

Berenji F, Rakhshandeh H, Ebrahimipour H (2010) *In vitro* study of the effects of henna extracts (*Lawsonia inermis*) on *Malassezia* species. Jundishapur J Microbiol 3:125–128

Boissier E (1844) Plantae aucherianae. Ann Sci Nat Bot 1:120–151

Borris RP (1996) Natural products research: perspectives from a major pharmaceutical company. J Ethnopharmacol 51:29–38

Clark AM (1996) Natural products as a resource for new drugs. Pharmaceut Res 13:1133–1141

Cowan MM (1999) Plant products as antimicrobial agents. Clin Microbiol Rev 12:564–582

Ebrahimabadi AH, Ebrahimabadi EH, Djafari-Bidgoli Z, Jookar-Kashi F, Mazoochi A, Batooli H (2010a) Composition and antioxidant and antimicrobial activity of the essential oil and extracts of *Stachys inflata* Benth, from Iran. Food Chem 119:452–458

Ebrahimabadi AH, Mazoochi A, Jookar-Kashi F, Djafari-Bidgoli Z, Batooli H (2010b) Essential oil composition and antioxidant and antimicrobial properties of the aerial parts of *Salvia eremophila* Bioss, from Iran. Food Chem Toxicol 48:1371–1376

Eftekhar F, Yousefzadi M, Azizian D, Sonboli A, Salehi P (2005) Essential oil composition and antimicrobial activity of *Diplotaenia damavandica*. Z Naturforsch 821–825

Firuzi O, Asadollahi M, Gholami M, Javidnia K (2010) Composition and biological activities of essential oils from four *Heracleum* species. Food Chem 122:117–122

Gandomi H, Misaghi A, Akhondzadeh-Basti A, Bokaei S, Khosravi AR, Abbasifar A, Jebelli-Javan A (2009) Effect of *Zataria multiflora* Bioss. Essential oil on growth and aflatoxin formation by *Aspergillus flavus* in culture media and cheese. Food Chem Toxicol 47:2397–2400

Ghahreman A (1995) Flora of Iran. Research Institute of Forest and Rangelands and Tehran University Press, Tehran

Ghasemi-Pirbalouti A, Bahmani M, Avijgan M (2009) Anti-*Candida* activity of some of the Iranian medicinal plants. Electron J Biol 5:85–88

Ghasemi-Pirbalouti A, Hamedi B, Abdizadeh R, Malekpoor F (2011) Antifungal activity of the essential oil of Iranian medicinal plants. J Med Plants Res 5:5089–5093

Ghorbanian M, Razzaghi-Abyaneh M, Allameh A, Shams-Ghahfarokhi M, Qorbani M (2007) Study on the effect of neem (*Azadirachta indica* A. Juss) leaf extract on the growth of *Aspergillus parasiticus* and production of aflatoxin by it at different incubation times. Mycoses 51:35–39

Gohari AR, Saeidnia S, Shahverdi AR, Yassa N, Malmir M, Mollazade K, Naghinejad AR (2009) Phytochemistry and antimicrobial compounds of *Hymenocrater calycinus*. EurAsian J BioSci 3:64–68

Kamrani A, Naqinezhad A, Attar F, Jalili A, Charlet D (2011) Wetland flora and diversity of the Western Alborz mountains, North Iran. Phytol Balcan 17:53–66

Khosravi AR, Shokri H, Kermani S, Dakhili M, Madani M, Parsa S (2011) Antifungal properties of *Artemisia sieberi* and *Origanum vulgare* essential oils against *Candida glabrata* isolates obtained from patients with vulvovaginal candidiasis. J Mycol Méd 21:93–99

Mahboubi M, Bidgoli G (2010) In vitro synergistic efficacy of combination of amphoteric in B with *Myrtus communis* essential oil against clinical isolates of *Candida albicans*. Phytomedicine 17:771–774

Mahboubi M, Kazempour N (2011) Chemical composition and antimicrobial activity of *Satureja hortensis* and *Trechyspermum opticum* essential oil. Iranian J Microbiol 3:194–200

Mohammadzadeh S, Shariatpanahi M, Hamedi M, Ahmadkhaniha R, Samadi N, Ostad SN (2007) Chemical composition, oral toxicity and antimicrobial activity of Iranian propolis. Food Chem 103:1097–1103

Morteza-Semnania K, Amin GH, Shidfar MR, Hadizadeh H, Shafiee A (2003) Antifungal activity of the methanolic extract and alkaloids of *Glaucium oxylobum*. Fitoterapia 74:493–496

Naeini A, Khosravi AR, Chitsaz M, Shokri H, Kamlnejad M (2009) Anti-*Candida albicans* activity of some Iranian plants used in traditional medicine. J Mycol Méd 19:168–172

Naeini AR, Nazeri M, Shokri H (2011) Antifungal activity of *Zataria multiflora*, *Pelargonium graveolens* and *Cuminum cyminum* essential oils towards three species of *Malassezia* isolated from patients with pityriasis versicolor. J Mycol Méd 21:87–91

Naeini AR, Ziglari T, Shokri H, Khosravi AR (2010) Assessment of growth-inhibiting effect of some plant essential oils on different *Fusarium* isolates. J Mycol Méd 20:174–178

Noorhosseini Niyaki SA, Ashoori Latmahalleh D, Allahyari MS, Doozandeh Masooleh P (2011) Socio-economic factors for adoption of medicinal plants cultivation in Eshkevarat region, North of Iran. J Med Plants Res 5:30–38

Nosrati S, Esmaeilzadeh-Hosseini SA, Sarpeleh A, Soflaei-Shahrbabak M, Soflaei-Shahrbabak Y (2011) Antifungal activity of spearmint (*Mentha spicata* L.) essential oil on *Fusarium oxysporum* f. sp. *radicis-cucumerinum* the causal agent of stem and crown rot of greenhouse cucumber in Yazd, Iran 2011. International conference on environmental and agriculture engineering, vol 15, pp 52–56

Omranpour M, Abbasi S, Bahraminejad S (2011) Evaluation of the inhibitory effect of some plant crude extracts against *Albugo candida*, the causal agent of white rust. World Acad Sci Eng Technol 58:362–364

Pasrsa A (1959a) Medicinal plants and drugs of plant origin in Iran I. Plant Foods Hum Nut 5:375–394

Pasrsa A (1959b) Medicinal plants and drugs of plant origin in Iran II. Plant Foods Hum Nut 6:69–96

Pasrsa A (1959c) Medicinal plants and drugs of plant origin in Iran III. Plant Foods Hum Nut 7:137–156

Pasrsa A (1960) Medicinal plants and drugs of plant origin in Iran IV. Plant Foods Hum Nut 7:65–136

Pauli A (2005) Hawaii Presentation Pacifi Chem 2005, part Ergosterol

Pauli A (2006) α-Bisabolol from Chamomile: a specific ergosterol biosynthesis inhibitor? Int J Aromather 16:21–25

Price M (2001) History of ancient medicine in Mesopotamia and Iran. http://www.iranchamber.com/history/articles/ancient_medicine_mesopotamia_iran.php

Ramezani M, Fazli-Bazzaz BS, Saghafi-Khadem F, Dabaghian A (2004) Antimicrobial activity of four *Artemisia* species of Iran. Fitoterapia 75:201–203

Rashidi A, Mousavi B, Rahmani MR, Rezaee MA, Hosaini W, Motaharinia Y, Davari B, Zamini G (2011) Evaluation of antifungal effect of *Lavandula officinalis*, *Salvia officinalis* L., *Sumac*, *Glycyrrhiza glabra*, and *Althaea officinalis* extracts on *Aspergillus niger*, *Aspergillus fumigatus*, and *Aspergillus flavus* species. J Med Plants Res 6:309–313

Rasooli I, Razzaghi-Abyaneh M (2004) Inhibitory effects of Thyme oils on growth and aflatoxin production by *Aspergillus parasiticus*. Food Control 15:479–483

Razzaghi-Abyaneh M, Allameh A, Tiraihi T, Shams-Ghahfarokhi M, Ghorbanian M (2005) Morphological alterations in toxigenic *Aspergillus parasiticus* exposed to neem (*Azadirachta indica*) leaf and seed aqueous extracts. Mycopathologia 159:565–570

Razzaghi-Abyaneh M, Shams-Ghahfarokhi M (2011) Natural inhibitors of food-borne fungi from plants and microorganisms. In: Rai M, Chikindas M (eds) Natural antimicrobials in food safety and quality. CABI Publisher, UK, pp 182–203

Razzaghi-Abyaneh M, Shams-Ghahfarokhi M, Rezaee MB, Jaimand K, Alinezhad S, Saberi R, Yoshinari T (2009) Chemical composition and antiaflatoxigenic activity of *Carum carvi* L., *Thymus vulgaris* and *Citrus aurantifolia* essential oils. Food Control 20:1018–1024

Razzaghi-Abyaneh M, Shams-Ghahfarokhi M, Rezaee MB, Sakuda S (2010) Natural aflatoxin inhibitors from medicinal plants. In: Rai M, Varma A (eds) Mycotoxins in food, feed and bioweapons. Springer, New York, pp 329–354

Razzaghi-Abyaneh M, Shams-Ghahfarokhi M, Yoshinari T, Rezaee MB, Nagasawa H, Sakuda S (2008) Inhibitory effects of *Satureja hortensis* L. essential oil on growth and aflatoxin production by *Aspergillus parasiticus*. Int J Food Microbiol 123:228–233

Rechinger K (1982) Flora Iranica. Graz, Austria

Sadeghi G, Abouei M, Alirezaee M, Tolouei R, Shams-Ghahfarokhi M, Mostafavi E, Razzaghi-Abyaneh M (2011) A 4-year survey of dermatomycoses in Tehran from 2006 to 2009. J Mycol Méd 21:260–265

Sadeghi-Nejad B, Shiravi F, Ghanbari S, Alinejadi M, Zarrin M (2010) Antifungal activity of *Satureja khuzestanica* (Jamzad.) leaves extracts. Jundishapur J Microbiol 3:36–40

Saei-Dehkordi SS, Tajik H, Moradi M, Khalighi-Sigaroodi F (2010) Chemical composition of essential oils in *Zataria multiflora* Bioss, from different parts of Iran and their radical scavenging and antimicrobial activity. Food Chem Toxicol 48:1562–1567

Sardari S, Amin G, Sekhon A, Micetich RG, Daneshtalab M (2000) Antifungal activity of *Diplotaenia damavandica*. Pharm Pharmacol Commun 6:455–458

Sarpeleh A, Sharifi K, Sonbolkar A (2009) Evidence of antifungal activity of wild rue (*Peganum harmala* L.) on phytopathogenic fungi. J Plant Dis Prot 116:208–213

Shams-Ghahfarokhi M, Goodarzi M, Razzaghi-Abyaneh M, Al-Tiraihi T, Seyedipour G (2004) Morphological evidences for onion-induced growth inhibition of *Trichophyton rubrum* and *Trichophyton mentagrophytes*. Fitoterapia 75:645–655

Shams-Ghahfarokhi M, Razafsha M, Allameh A, Razzaghi-Abyaneh M (2003) Inhibitory effects of aqueous onion and garlic extracts on growth and keratinase activity in *Trichophyton mentagrophytes*. Iranian Biomed J 7:113–118

Shams-Ghahfarokhi M, Shokoohamiri MR, Amirrajab N, Moghadasi B, Ghajari A, Zeini F, Sadeghi G, Razzaghi-Abyaneh M (2006) *In vitro* antifungal activities of *Allium cepa, Allium sativum* and ketoconazole against some pathogenic yeasts and dermatophytes. Fitoterapia 77:321–323

Sharafzadeh S, Alizadeh O (2012) Some medicinal plants cultivated in Iran. J Appl Pharmaceut Sci 2:134–137

Stockwell C (1988) Nature's Pharmacy. Century Hutchinson Ltd., London

Tolouee M, Alinezhad S, Saberi R, Eslamifar A, Zad SJ, Jaimand K, Taeb J, Rezaee MB, Kawachi M, Shams-Ghahfarokhi M, Razzaghi-Abyaneh M (2010) Effect of *Matricaria chamomilla* L. flower essential oil on the growth and ultrastructure of *Aspergillus niger* van Tieghem. Int J Food Microbiol 139:127–133

WHO (2002) WHO traditional medicine strategy 2002–2005. World health report, World Health Organization, Geneva

Yadegarinia D, Gachkar L, Rezaei MB, Taghizadeh M, Astaneh SA, Rasooli I (2006) Biochemical activities of Iranian *Mentha piperita* L. and *Myrtus communis* L. essential oils. Phytochemistry 67:1249–1255

Yazdani D, Rezazadeh SH, Amin GH, Zainal-Abidin MA, Shahnazi S, Jamalifar H (2009) Antifungal activity of dried extracts of anise (*Pimpinella anisum* L.) and star anise (*Illicium verum* Hook. f.) against dermatophyte and saprophyte fungi. J Med Plants 5:24–29

Zargari A (1992) Medicinal plants. Tehran University Press, Tehran

Chapter 3
Antifungal Property of Selected Nigerian Medicinal Plants

Victor Olusegun Oyetayo and Ayodele Oluyemisi Ogundare

Abstract The effectiveness of common antimicrobial substances to microbial entities has been in doubt in the last three decades. This is as a result of resistance of microbial spoilers and pathogens to these antimicrobial agents. Fungi which are microbial entities are not exempted from the problem of antimicrobial resistance. Most common fungicidal agents are becoming ineffective in treating fungal infections. Hence, the search for antifungal agents has double paced recently. Plants that have the potential to synthesize aromatic substances with antimicrobial properties have been proposed as the sources from which novel and effective antimicrobial substances can be derived. This chapter X-rays the resistance of fungi to common antifungal agents and the possibility of using bioactives from plants to solve the increasing problem of fungal resistance.

3.1 Introduction

In the last two to three decades, there has been a renewed search for antimicrobial agents that are effective and safe. The search has been necessitated based on the fact that microorganisms had developed resistance to commonly used antimicrobial agents and also the issue of safety of some of the available antimicrobial agents. The side effects associated with the use of antibiotics in the treatment of gastroenteritis and other infections definitely necessitated the search for a suitable and friendlier alternative.

Fungi which are microbial entities are not exempted from the problem of antimicrobial resistance. Most fungi have developed resistance to commonly available fungicidal agents. For instance, the broad-spectrum drug amphotericin B

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was the sole drug for nearly 30 years, and it is one of the few drugs that actually kill fungal cells, but can cause significant nephrotoxicity in the patients (Arif et al. 2009). One group of antifungal agents is the azole group. The question of fungal resistance to the azole drugs is considerably more complex and is currently under evaluation (Como and Dismukes 1994). For instance, pathogenic fungus, *Candida albicans* strains have been reported to be susceptible to fluconazole and other azole antifungals; however, increasing resistance especially in HIV-infected hosts has been observed in hosts undergoing repeated courses of azole antifungal therapy (Iwata 1992).

Synthetic chemicals such as Blitox, Captan, Dithane M-45, and Thiram used in controlling plant diseases and preventing seed biodeterioration during storage are known to have residual toxicity and nonbiodegradable in nature and hence can cause carcinogenicity, teratogenicity on nontarget organisms and pollute the environment, soil, and ground water (Pimentel and Levitan 1986; Satish et al. 2009). Therefore, there is restriction on the use of many of the synthetic fungicides because of their undesired attributes, such as high and acute toxicity, long degradation period and accumulation in the food chain and an undesirable extension of their power to destroy useful microorganisms (Satish et al. 1999).

Plants have a long history of antibiotic usage for the cure of disease caused by microbial agents, including viral, bacterial, and fungal agents (Bhadauria and Kumar 2011). Plants have been described as the sleeping giants that possess the bioactive agents that can solve the problem of antibiotic resistance which is currently a major global issue. Several researchers have reported the antifungal activities of some plants (Caceres et al. 1993; Mehrabian et al. 1995; Farombi 2003; Oyelami et al. 2003; Nair et al. 2005; Mahesh and Satish 2008; Prusti et al. 2008).

Despite the efforts of researchers in screening plants for their antifungal property, there are still so many plants that are yet to be assessed for their antifungal property and this require more careful investigation to reveal their hidden characteristics (Bhadauria and Kumar 2011). Humans have utilized relatively 1–10 % of the estimated 250,000–500,000 species of plants on Earth (Brinker 1993). In essence, 90 % of these plants are yet to be utilized. Specifically, a lot of these yet to be investigated plants with potential antifungal properties are still in the wild in Nigeria and most West African Countries. Scientific documents of the identities and antifungal properties of these plants are still scanty. Natural products have been the single most productive source of leads for the development of drugs (Harvey 2008). Generally, natural products from plants are more preferred, because they are harmless or have minimum side effects when compared to synthetic drugs.

Fungi are eucaryotes and are more evolutionarily advanced forms of microorganism. They are genetically more complex than prokaryotes (prions, viruses, and bacteria). Their genetic complexity had resulted in complex structural features that are used in classifications. Fungi can be classified into two, yeasts and molds, based on their morphological features. Molds form hyphal and a mass of hyphal is referred to as mycelium. Some fungi are able to exhibit dimorphism, that is, to

exist either as yeast or hyphal. Molds are achlorophyllus fungi and they exist as heterotrophs, that is, they live on preformed organic matter.

Fungi are widely spread and this has made infection caused by these entities frequent (Fleming et al. 2002). The pathogenic effect of fungi can be confirmed by the Koch postulate. This involves isolating the suspected fungus from a serial specimens and the fungus isolated must be consistent in morphology with the isolate observed in infected tissue(s).

There are some obvious challenges in application and evaluation of fungicidal agents in the treatment of infection caused by fungi. Fungi are eukaryotes, and consequently most agents toxic to fungi are also toxic to the host; unlike bacteria which are prokaryotic and hence offer numerous structural and metabolic targets that differ from those of the human host. It also known that fungi generally grow slowly and often in multicellular forms, hence, they are more difficult to quantify than bacteria. Inability to easily quantify fungi has complicated experiments designed to evaluate the in vitro or in vivo properties of a potential antifungal agent.

3.2 Resistance of Fungi to Common Antifungal Agents

The attention of researchers on antifungal drug has not been as serious as what is observed for bacteria and viruses. This is obvious as demonstrated by the fact that the amphotericin B, a polyene antibiotic discovered as long ago as 1956, is still used as antifungal agent (Abad et al. 2007). Antifungal agents are drugs that selectively eliminate fungal pathogens from a host with minimal toxicity to the host. These agents can be fungistatic or fungicidal. Effective antifungal agent must possess fungicidal property (ability to kill the fungal cell).

Antifungal agents are sourced from these groups of drugs: Polyene (nystatin, amphotericin B, and pimaricin), Azole (imidazoles are clotrimazole, miconazole, and ketoconazole), Allylamine (naftifine, terbinafine), Morpholine (amorolfine), and Antimetabolite such as 5-Fluorocytosine which acts as an inhibitor of both DNA and RNA synthesis via the intracytoplasmic conversion of 5-fluorocytosine to 5-fluorouracil. These antifungal agents have different modes of action. Polyene antifungal drugs, for instance, interact with sterols in the cell membrane (ergosterol in fungi, cholesterol in humans) to form channels through which small molecules leak from the inside of the fungal cell to the outside. Azole group of antifungal drugs, on the other hand, inhibits cytochrome P_{450}- dependent enzymes (particularly C14-demethylase) involved in the biosynthesis of ergosterol, which is required for fungal cell membrane structure and function. Allylamine/morpholine and antimetabolite antifungal agents inhibit ergosterol biosynthesis at the level of squalene epoxidase and acts as an inhibitor of both DNA and RNA synthesis via the intracytoplasmic conversion of 5-fluorocytosine to 5-fluorouracil, respectively.

There is an increasing problem of antifungal drug resistance even with the development of more antifungal agents. The two drawbacks of antifungal drugs are the development of fungal resistance and toxic side effects. Two common

groups of antifungal agents, imidazoles and the triazoles which act by inhibiting processes of the fungal cell have been found to result in recurrence of the infection and the development of resistance to the drug (Rex et al. 1995). The issue of toxicity and nonbiodegradability of some of these currently available antifungal agents calls for alternative agents. It is therefore expedient to search for new, safer, and more potent agents to combat serious fungal infections of plants and animals (Arif et al. 2009).

3.3 Fungi Pathogenic to Plants and Animals

Fungi are etiological agents implicated in several plants and animal diseases. Stored food crops are usually spoiled by fungi. Plants in field are not also spared from the pestiferous activity of fungi. Some of the field fungi are usually transported to storage facilities where they cause spoilage of stored products. Members of the genera Aspegillus, Fusarium, and Penicillium have been implicated in the spoilage of grains, fruits, vegetables, and other plant products during picking, transit, and storage rendering them unfit for human consumption by producing mycotoxins and affecting their nutritive value (Miller 1995; Janardhana et al. 1998; Galvano et al. 2001). Other species of fungal genera of importance in the spoilage of agricultural produce are *Colletorichum coccodes*, *Botrytis cinerea*, *Xanthomonas campetris Pseudomonas syringae*, *Alternaria alternata*, and *Phytophthora drechsleri*. The overall effects of these fungi activities lead to lower economic value of agricultural products and make them unfit for human consumption.

Humans and animals are not exempted from fungal infection. Fungal related diseases may not be as common as other microbial infections in animals but, when present, they are difficult to treat especially in immunosuppressed persons (Bryce 1992). Fungal infections have contributed to the increase of life-threatening systemic fungal infections in recent years (Perea and Patterson 2002). Some of the factors responsible for these are: the expansion of severely ill and/or immunocompromised patient population, including HIV-infected patients, patients with cancer who have chemotherapy-induced neutropenia, and transplant recipients who are receiving immunosuppressive therapy (Beck-Sague et al. 1993; Perea and Patterson 2002). Individuals with weakened immune system are more susceptible to fungal infections. Hostettmann and Marston (1994) listed the commonly observed infections in the immuno-compromised host to include candidiasis (*Candida albicans* and other species) of the esophagus and mouth, cryptococcosis (*Cryptococcus neoformans*), and aspergillosis (*Aspergillus flavus,* A. *fimigatus, and A. niger*).

Infection caused by fungi is known as mycosis. Mycoses can be classified based on the site of infection in human. Superficial infections are infections of the outer layer of the stratum corneum of the skin. Cutaneous infections involve the infections of the hair, skin, and nails while subcaneous and deep mycoses are the infections of the inner tissues and infections caused by primary and opportunistic

fungal pathogens, respectively (Odds et al. 1992; Rinaldi and Dixon 1994). Superficial fungal infections are common in most tropical areas and in developing countries. The factors responsible for this include the hot climatic conditions and high humidity coupled with malnutrition and overcrowding (Oyelami et al. 2003).

Some common fungi involved in human and animal mycoses are: superficial mycoses (*Piedraia hortae*, *Trichosporon beigelii*, and *Malassezia furfur Phaeoannellomyces werneckii*); cutaneous mycoses (*Candida* spp., *Epidermophyton floccosum, Trichophyton* spp.); and subcutaneous mycoses (*Fonsecaea pedrosoi*, *Fonsecaea compacta*, *Cladosporium carionii*, *Phialophora verrucosa*, *Nocardia brasiliensis*, and *Sporothrix schenckii*).

3.4 Antifungal Compounds from Plants

Natural products are made up of several chemical compounds and hence possess great potentials in the discovery of new and probably more effective pharmaceuticals. Researchers had turned to natural products from plants, microbes, and animals as sources of new pharmaceuticals for treating various ailments. Harvey (2008) listed some compounds from natural products to include elliptinium, galantamine, and huperzine from plants; daptomycin from microbes and exenatide and ziconotide from animals, as well as synthetic or semi-synthetic compounds based on natural products (e.g., tigecycline, everolimus, telithromycin, micafungin, and caspofungin). Natural products had been very important in drug discovery and were the basis of most early medicines (Sneader 1996; Newman et al. 2000; Buss et al. 2003). Development in the field of analytical chemistry over the last 10–15 years with advances in X-ray crystallography, nuclear magnetic resonance (NMR), and alternative drug discovery methods such as rational drug design and combinatorial chemistry have placed great pressure upon natural product drug discovery programmes (Butler 2005; Newman et al. 2000).

Reports show that most antifungal drugs in use today have some connection to natural products (Butler 2005). The polyenes and griseofulvin are natural products, while the echinocandins are semi-synthetically derived from natural products. 5-Fluorocytosine is a nucleoside that interferes with DNA and RNA synthesis and is primarily used in combination with the polyene amphotericin B (Butler 2005). Sneader (1996) traced the origin of azole, generally considered to be synthetic in origin, to drug prototype pathway in Streptomyces metabolite, azomycin.

Siva et al. (2008) stated that higher plants possess bioactives which are active against fungi and could be used in disease control. These compounds possess two important characteristics. They are biodegradable and also selectively toxic, hence of value in the control of plant disease (Siva et al. 2008). Bioactives in plants can serve two purposes; they may be the base for the development of new drugs or, a phytomedicine in the treatment of diseases (Iwu 1993). Approximately 25 % of the active substance prescriptions in the United States come from plant material (Céspedes et al. 2006).

Plants have an almost limitless ability to synthesize aromatic substances of different functional groups, most of which are phenols or their oxygen-substituted derivatives (Cowan 1999; Arif et al. 2009). About 12,000 of these aromatic substances and their derivatives have been isolated and this represents roughly 10 % of the total (Schultes 1978; Cowan 1999). Plants use these metabolites for defense against predators such as microorganisms, insects, and herbivores. These compounds are also responsible for plant odors (terpenoids) and pigments (quinones and tannins).

Some major groups of antifungal compounds found in plants are listed below.

3.4.1 Crude Extracts

Crude extracts could be infusion or decoction from medicinal plants. These extracts are made up of 10–1,000 different bioactive compounds. Their activities in many cases are as a result of synergy between 2 or more of the bioactives present in extracts. Crude extracts had been used in various parts of the world to treat fungal infection. Bergeron et al. (1996) reported the antifungal activities of crude extract of 19 plant species from 14 families used in traditional North American Indian medicine against *Cladosporium cucumerinum* and *C. albicans*. Researchers in Europe, Asia, Africa, Mexico, and Brasil had also reported antifungal activities of extracts of medicinal plants on various pathogenic fungi (Verastegui et al. 1996; Tadeg et al. 2005; Turchetti et al. 2005; Lamidi et al. 2005).

3.4.2 Phenolic Compounds

Phenolics constitute one of the major groups of nonessential dietary components in plants. There are two types of phenolics, the nonsoluble phenols such as tannins, lignins, cell-wall bound hydrocinammic acids, and soluble phenols such as phenolic acids, phenylpropanoids, flavonoids, and quinines (Rispail et al. 2005). Phenolic acids possess potential protective role, through ingestion of fruits and vegetables, against oxidative damage diseases (coronary heart disease, stroke, and cancers). They are essential for the growth and reproduction of plants, and are produced as a response for defending injured plants against pathogens. Some phenolic classes with antifungal properties found in medicinal plants are simple phenolic compounds, flavones and related flavonoid glycosides, coumarins and derivatives, and anthraquinones (Abad et al. 2007). Phenolic compounds such as tannins present in plant cells are potent inhibitors of many hydrolytic enzymes such as pectyolytic macerating enzymes used by plant pathogens (Cowan 1999). These compounds had been reported to have exhibit good antioxidant property. Three new phenolic compounds

isolated from the leaves of *Baseonema acuminatum* P. Choux (Asclepiadaceae) showed antifungal activity against two clinically isolated *C. albicans* strains with IC50 values in the range of 25–100 μg/ml (De Leo et al. 2004). Four phenolic acid derivatives were isolated from an ethyl acetate extract of the root bark of *Lycium chinense* Miller (Solanaceae). All had antifungal effect and inhibited the dimorphic transition of the pathogen, *C. albicans*. Flavonoid compounds from spices of Fabaceae and Moraceae have been reported to have antifungal activity (Abad et al. 2007). Some of these flavonoids were isolated by bioassay-guided fractionation, after previously detecting antifungal activity on the part of the plant.

3.4.2.1 Saponins

Saponins are secondary metabolites that occur in a wide range of plant species. They are stored in plant cells as inactive precursors but are readily converted into biologically active antibiotics by enzymes in response to pathogen attack (Cowan 1999; Arif et al. 2009). Saponnins can be divided into three major groups; a triterpenoid, a steroid, or a steroidal glycoalkaloid (Arif et al. 2009). Two new steroidal saponins isolated from the roots of *Smilax aspera* subsp. *mauritanica* exhibited antifungal activity against the human pathogenic yeasts, *C. albicans*, *C. glabrata*, and *C. tropicalis* in the range of 25–50 mg/ml (Belhouchet et al. 2008). Other saponins such as Phytolaccosides B and E from *Phytolacca tetramera* showed antifungal activities against a panel of human pathogenic opportunistic fungi (Escalante et al. 2002); Tigogenin-3-O-b-D-xylopyranosyl-(1-2)-[b-D-xylopyranosyl-(1-3)]-b-D-glucopyranosyl-(1-4)-[a-L-rhamnopyranosyl-(1-2)]-b-D-galactopyranoside was found to have in vivo activity in *C. albicans* vaginal infection model (Zhang et al. 2005). Tigogenin-3-O-b-D-glucopyranosyl-(1-2)-[b-D-xylopyranosyl-(1-3)]-b-D-glucopyranosyl-(1-4)-b-D-galactopyranoside was found to be in vitro very effective against several pathogenic *Candida* species.

3.4.2.2 Alkaloids

Alkaloids are heterocyclic nitrogen compounds. They are a large group of substances that occur naturally in plants and usually have a bitter taste. Alkaloids protect plants against pest and microorganisms.

Some common examples include caffeine, morphine, nicotine, quinine, and strychnine. Morphine was the first medically useful alkaloid isolated in 1805 from the opium poppy, *Papaver somniferum* (Facchini et al. 1996). Several alkaloids such as 2-(3, 4-dimethyl-2,5-dihydro-1H-pyrrol-2-yl)-1-methylethyl pentanoate isolated from the plant *Datura metel*; 6,8-didec-(1Z)-enyl-5,7-dimethyl-2,3-dihydro-1H-indolizinium from *Aniba panurensis*; Bromo-8-n-hexylberberine, a derivative of berberine have been reported to have antifungal activities (Arif et al. 2009).

3.4.2.3 Peptides and Proteins

The antifungal properties of peptides and proteins isolated from medicinal plants have been isolated from species of the Fabaceae family. Wong and Ng (2005) purified an antifungal peptide named vulgarinin from the seeds of haricot beans, *Phaseolus vulgaris* L. (Fabaceae) which display antifungal activity toward fungal species such as *Fusarium oxysporum*, *Mycosphaerella arachidicola*, *Physalospora piricola,* and *Botrytis cinerea*. There has been report of other antifungal agents such as chitinase isolated from mung bean (*Phaseolus mungo* L.) seeds (Wang et al. 2005), defensins isolated from *Trigonella foenum-graecum* L., (Olli and Kirti 2006), AFP-J purified from potato tubers, *Solanum tuberosum* cv L. Jopung (Solanaceae) (Park et al. 2005), and a thaumatin-like protein from banana *Musa acuminata* Colla (Musaceae) (Wang and Ng 2005).

3.5 Some Selected Nigerian Plants with Antifungal Properties

Nigeria with her geographic spread from the Northern Savannah to the Southern forest is a home to diverse kinds of medicinal plants. Most of these plants are still in the wild. The antifungal properties of some of these plants reported by several authors are listed below.

3.5.1 Brysocarpus coccineus Schum and Thonn

This plant belongs to the family Connaraceae. It is a climbing shrub and native to Africa. Traditionally, there are reports that the leaf of the plant is used in treating ailments such as veneral diseases, impotency, diarrhea, jaundice, piles, dysentery, earache, sore of mouth and skin, tumor, wounds, stomatitis, rheumatism, swellings, and urinary disorders (Dalziel 1955; Burkill 1985; Akindele and Adeyemi 2006). In a recent study, Hamid and Aiyelaagbe (2010) reported that ethylacetate and methanol extracts of the stem of *Brysocarpus coccineus* exhibited antifungal properties against *Rhizopus stolonipher* and *Epidermophyton floccosum*, while hexane also inhibited the growth of *Rhizopus stolonipher, Epidermophyton floccosum, Tricophyton rubrum,* and *Aspergillus niger* with activity comparable to that of the reference drug tioconazole. Preliminary phytochemical screening of the three extracts revealed the presence of saponins, reducing sugar, steroids, glycosides, flavonoids, and anthraquinones (Hamid and Aiyelaagbe 2010).

3.5.2 *Ocimum gratissimum*

This plant belongs to the family lamiaceae. There are 20–150 *Ocimum* species spread across Asia, Africa, Central and South America (Darrah 1980). *Ocimum* is very rich in aromatic compounds and essential oils. Bioactives present in extracts from *Ocimum* had been reported to possess insecticidal, nematicidal, fungicidal, and generally antimicrobial (Morales and Simon 1996). Antifungal activity of Ocimum extract on *Aspergillus repens*, *Curvularia lunata,* and *Fusarium moniliforme* was reported by Amadi et al. (2010). Water, ethanol, and acetone extracts of ocimum impaired radial growth of the three fungi, *A. repens*, *C. lunata,* and *F. moniliforme*. Phytochemical screening of *Ocimum* extracts in this study showed that carbohydrates, reducing sugars, lipids, alkaloids, and steroids tannins (Amadi et al. 2010).

3.5.3 *Acalypha wilkesiana* (Muell Arg.)

Acalypha wilkesiana belongs to the Euphorbiaceae family. Oliver (1959) reported that the leaf of *Acalypha wilkesiana* is used for treating *Pityriasis versicolor*, *Tinea versicolor,* and other similar skin infections. Antimicrobial activity of *Acalypha* species has been linked to the presence of phytochemical such as geranin, corilagin, and gallic acid (Adesina et al. 2000). Polyphenols such as ellagitannins, acalyphidins M1 and M2, and a dimer of geranin, calyphidin D1 have also been isolated from *A. hispida* (Yoshiaki et al. 1999). In a study conducted by Oyelami et al. (2003), it was observed that *A. wilkesiana* ointment was very efficacious in the treatment of *Tinea pedis*, *Pityriasis versicolor,* and *Candida intetrigo* where the cure rate was 100 % in each condition.

3.5.4 *Anogeissus leiocarpus (DC.)* Guill and Perr

Anogeissus leiocarpus is a tall evergreen tree native to savannas of Tropical Africa. It belongs to the family, Combretaceae. It is also used in traditional medicine as a remedy for many ailments of livestock and man, which include helminthosis, schistosomiasis, leprosy, diarrhea, and psoriasis (Burkill 1985; Onyeyili 2000). Mann et al. (2008) reported the antifungal activity of chloroform, ethanolic, methanolic, ethyl acetate, and aqueous root extracts of *Anogeissus leiocarpus* against *A. niger*, *Aspergillus fumigatus*, *Penicillium* species, *Microsporum audouinii,* and *T. rubrum* using radial growth technique.

3.5.5 *Terminalia avicennoides* Guill and Perr

Terminalia avicennoides is a tree that is abundant in the Savannah region of West Africa. Ethnomedicinal uses of the plant cover various medical conditions. Abdullahi et al. (2001) reported that the powered roots are used for treating wounds and ulcers and a root decoction is used for the treatment of gastrointestinal disorders. In a study using radial growth technique, Mann et al. (2008) observed that chloroform, ethanolic, methanolic, ethyl acetate, and aqueous root extracts of *Terminalia avicennoides* have antifungal activities against *A. niger*, *A. fumigatus*, *Penicillium* species, *Microsporum audouinii,* and *T. rubrum*. The minimum inhibitory concentration (MIC) of the extracts ranged between 0.03 and 0.07 μg/ml while the minimum fungicidal concentration ranged between 0.04 and 0.08 μg/ml (Mann et al. 2008). The major phytochemicals present in *T. avicennoides* are saponins, tannins, and glycosides (Aboaba et al. 2006).

3.5.6 *Funtumia elastica* (Preuss)

Funtumia elastica is a medium-sized African rubber tree with glossy leaves, milky sap, and long woody seedpods. It belongs to the family Apocynaceae. The genus consists of two common species: *F. elastica* (female) and *F. africana* (male) (Adekunle and Ikumapayi 2006). The decoction of the leaf is used as a cure for mouth and venereal diseases (Sofowora 1982). Phytochemical screening of *F. elastic* revealed that it contains anthocyanins, butacyanin, flavonoids, steroids, and tannins. *F. elastica* also exhibited pronounced antifungal activity *Aspergillus flavus, C. albicans, Microsporum audouinii*, *Penicillium* sp., *Trichophyton mentagrophytes*, *Trichoderma* sp, *and Trichosporon cutaneum* (Adekunle and Ikumapayi 2006).

3.5.7 *Mallotus oppositifolius* (Geisel) Müll. Arg

Mallotus oppositifolius belongs to the family Euphorbiaceae. The twig of the plant is used as chewing sticks for cleaning the teeth, the stem is used as yam stakes. Phytochemical screening of *M. oppositifolius* revealed the presence of secondary metabolites such as alkaloids, phenols, flavonoids, anthraquinones, and cardenolides. Higher concentration of these phytochemicals resides in the leaves than in the root (Farombi et al. 2001). The leaves of *M. oppositifolius* are used in preparing antimalarial and antiinflammatory remedies (Burkhill 1994). The antifungal property of aqueous and ethanol extracts of *M. oppositifolius* against fungi such as *A. flavus, C. albicans, Microsporum audouinii, Penicillium* sp., *T. mentagrophytes, Trichoderma* sp. *and Trichosporon cutaneum* was reported by Adekunle and Ikumapayi (2006).

3.5.8 *Asystasia gangetica* (Linn) T. Anders.

Asystasia gangetica belongs to the family Acanthaceae. It is an ascending, branched, quadrangular fast growing plant with stem up to 2 m long and roots at lower nodes (Ensermu 1994). The hexane, ethylacetate, and methanol extracts showed antifungal activities against *C. albicans*, *Penicillium notatum*, *T. rubrum,* and *Epidermophyton floccosum* with activity comparable to that of the reference drug, Tioconazole (Hamid et al. 2011). The MIC of the plants against the fungi ranged from 6.25 to 200 mg/ml. Extracts of this plant contain saponins, reducing sugar, steroids, glycosides, flavonoids, and anthraquinones (Hamid et al. 2011).

3.5.9 *Carica papaya Authority*

Carica papaya belongs to the family Caricaceae. It is a cultivated tropical fruit tree. Pawpaw is the fruit of the plant. Previous report shows that Papaya seed could be used as an antibacterial agent for *Escherichia coli, Staphylococcus aureus,* and *Salmonella typhi* (Yismaw et al. 2008). Methanol extracts of leaves of Carica papaya at 20, 40, 60, and 80 % concentrations had been reported to possess fungitoxic components that retarded the mycelia growth of *Alternaria solani*, isolated from rotting yam tubers (Suleiman 2010).

3.5.10 *Lemna pauciscostata* (Helgelm)

Lemna, a group of tiny, free-floating vascular plants with worldwide distribution are found in small water bodies such as fishponds, ditches and lagoons, which are nutrient rich (Effiong and Sanni 2009). Earlier study had shown that Lemna possess antimicrobial property (Skilicorn et al. 1993; Mbagwu 2001). In a study, Effiong and Sanni (2009) reported the antifungal effect of Lemna against *Fusarium oxysporium*, *Penicillium digitatum*, *A. niger*, *A. flavus*, *A. fumigatus*, *Rhizopus oryzae, and R. stolonifer*. Ethanolic extracts exhibited higher antifungal properties with total growth inhibition in some test organisms than the aqueous extract. Phytochemical screening revealed the presence of tannins and steroids in duckweed (Effiong and Sanni 2009).

3.5.11 *Diodia scandens* Sw

Diodia scandens belongs to the family Rubiaceae. It is a straggling herb with a taproot, slender stem, and compound leaves used for treatment of various diseases

(Essiett et al. 2010). *D. scandens* has a lot of medicinal uses. In Nigeria, the leaves are used for curing eczema (antifungal property), as fodder to poultry, its juice is used to stop bleeding, its extract is used to treat bruises and minor cuts and drunk as tonic during treatment of ear problems, it is also used as antiabortifacient (Etukudo 2003). Phytochemical screening of the leaf of *D. scandens* revealed the presence of saponins, tannins, cardiac glycosides and absence of flavonoids, phlobatannins, alkaloids, and anthraquinones (Essiett et al. 2010).

3.5.12 Senna alata Linn

Senna alata belongs to the family Fabaceae. It is an ornamental shrub, which grows well in forest areas of West Africa (Owoyale et al. 2005). *S. alata* plants contain a group of phytochemicals like saponin, alkaloid, steroid, flavonoid, tannin, phenol, and carbohydrate (Akinyemi et al. 2000). Stem bark of *S. alata* is used to treat fungal infections such as ringworm. It is a common ingredient in soaps, shampoos, and lotions because of its antifungal properties (Sule et al. 2011). Crude extract of the stem bark of *S. alata* exhibited marked antifungal effects on *Microsporum canslaslomyces*, *Trichophyton verrucosum*, *T. mentagrophytes*, and *Epidermophyton floccosum*. The crude extract showed the highest inhibition on *T. verrucosum* and *E. floccosum* with 21.00 and 20.05 mm zones of inhibition, respectively (Sule et al. 2011). Phytochemical analysis of the stem bark of *S. alata* revealed the presence of tannins, steroids, alkaloids, anthraquinones, terpenes, carbohydrates, and saponins (Sule et al. 2011).

3.5.13 Calotropis procera Ait. f

This plant belongs to the family Asclepiadaceae. It is commonly called Sodom apple. It is un-branched with soft wooden trunk, yellowish brown stem bark and the slash exudes caustic latex that turns yellow on exposure to air (Aliero et al. 2001). The juice from the plant is used in cuddling milk in the production of a local milk product called ***wara***. Ethnomedicinal uses of the plant include the treatment of fungal diseases, convulsion, asthma, cough, and inflammation (Hassan et al. 2006). Organic solvents and aqueous extracts of the stem bark, leaves, and roots were found to significantly inhibit common dermatophytes such as *T. rubrum* and *Microsporum gypseum*, and a common spoilage fungus, *A. niger* (Hussain and Deeric 1991). Alkaloids, flavonoids, tannins, steroids, triterpenoids saponins, and saponin glycosides have been reported in the leaves and roots extracts while stem bark contains flavonoids, triterpenoids, and saponins (Hussain and Deeric 1991).

3.6 Other Indigenous Nigerian Plants with Antifungal Property

Researchers in Nigeria had assessed the antifungal property of several other plants indigenous plants. A list of some of these plants is presented below in Table 3.1. The list, however, is not exhaustive.

3.7 Future Perspectives

Antifungal agents sourced from plants hold the wand in solving the problem of resistance to antifungal agent. Renewed efforts to screen yet to be explored plants for their potential antifungal properties should be intensified. Most research reports on antifungal potentials of Nigerian plants were assessed using extracts. Extracts contain ten to hundreds of compounds. The observed antifungal effect may be as a result of synergy between two or more bioactive agents present in the extract. It is therefore important to isolate and identify the specific bioactive compound(s) responsible for the expressed antifungal property observed in the plants. Extracts and their isolated/identified bioactive compounds assessed by in vitro techniques should also be verified using in vivo techniques. This will help to know if what is observed outside a living system can be replicated in a living system. A well-structured in vivo study will determine the effectiveness of such antifungal agent in whole organism systems. In the search for antifungal agent, attention should be focused on fungicidals that are nontoxic to the host rather than fungistatic agents.

Molecular farming is also desirable to increase the productivity and efficacy of medicinal plants in the production of bioactives of interest. Molecular biological techniques will bring about the development of novel antifungal agent that will be safe and effective. Plants have the advantages: Can be grown under conditions of biological and physical containment e.g., green house; do not carry most human disease pathogens, hence reducing the risk of drugs being contaminated with animal pathogens; can be transformed to produce a wider variety of novel and more suitable bioactive compounds and at higher concentration. Moreover, structural modification of the existing plant products is also expedient to increase their activity. Isodilapiole tribromide, a derivative of dillapiole was found to be more active than the initial compound. Similarly, echnicandin-type peptide FR901379, chemical modification led to the formation of more active FK463 compound (Arif et al. 2009).

Table 3.1 Nigerian plants with antifungal property

Plant	Family	Part(s) used	Susceptible fungí	Reference
Sphenoceutrum jollyanum	Menispermaceae	Root (methanol extract)	C. albicans, *C. pseudotropicalis, T. rubrum*	Aladesanmi et al. (2007).
Trichilia heudelotti	Meliaceae	Leaf (methanol extract)	*C. albicans, C. pseudotropicalis, T. rubrum*	Aladesanmi et al. (2007)
Aframumum melegueta	Zingiberaceae	Seed	*A. niger, A. flavus, P. notatum.*	Ayodele et al. (2009).
Chrysanthellum americanum	Asteraceae	Leaf	*C. albicans, T. rubrum, P. notatum, C. tropicalis*	Ofodile et al. (2010).
Cocos nucifera	Asteraceae	Coconut oil	*C. albicans, C. krusei, C. glabrata, C. parapsilosis, C. stellatoidea*	Ogbolu et al. (2007).
Ageratum conyzoides	Asteraceae	Leaf	*Corynespora cassiicola*	Ogbebor and Adekunle (2005)
Alafia barteri Oliv.	Apocynaceae	Stem	*C. albicans, Aspergillus niger, Rhizopus stolon, Penicillum notatum, Tricophyton rubrum, Epidermophyton floccosum*	Hamid and Aiyelaabge (2011)
Xylopia aethiopica	Annonaceae	Fruit/pod	*T. rubrum*	Henley et al. (2007)
Plumbago zeylanica	Plubagiaceae	Leaf and root	*C. albicans*	Ogbebor and Adekunle (2005)
Vernonia tenoreana	Compositae	Bark	*C. albicans*	Ogundare et al. (2006)
Acalypha fimbriata	Euphorbiaceae	Leaf	*C. albicans*	Adodo (2005)
Senna podocarpa	Leguminosae	Leaf	*C. albicans*	Ogundare (2009)

3.8 Conclusion

Natural products of plant origin still remain the main source of novel compounds that can solve the problem of drug resistance posed to currently available drugs by microbial agents. Concerted effort should be geared toward screening the about 90 % of plants whose activities are still unknown for their antifungal property. The yet to be screened plants are therefore a source to search for new chemotypes for drug development in antifungal therapeutics.

Antifungal agents that are currently available are costly and therefore unaffordable to poor peasants in the developing world. Plant extracts, on the other hand, are affordable, safer, and easily available to common men. Plants are source of thousands of new useful phytochemicals of great diversity, which have inhibitory effects on all types of microorganisms in vitro. Application of plant products in integrated pest management may reduce over reliance on one source of agricultural chemical to the farmer, as well as cut down cost of production.

References

Abad MJ, Ansuategui M, Bermejo P (2007) Active antifungal substances from natural sources. Arkivoc 7:116–145

Aboaba OO, Smith SI, Olude FO (2006) Antibacterial effect of edible plant extract on *Escherichia coli* 0157:H7. Pakistan J Nutr 5(4):325–327

Abdullahi AL, Agho MO, Amos S, Gamaniel KS, Wambebe C (2001) Antidiarrhoeal activity of the aqueous extract of *Terminalia avicennoides* roots. Phytother Res 15:431–434

Adekunle AA, Ikumapayi AM (2006) Antifungal property and phytochemical screening of the crude extracts of *Funtumia elastica* and *Mallotus oppositifolius*. West Indian Med J 55(4):219–223

Adesina SK, Idowu O, Ogundaini AO, Oladimeji H, Olugbade TA, Onawunmi GO, Pais M (2000) Antimicrobial constituents of the leaves of *Acalypha wilkesiana* and *Acalypha hispida*. Phytother Res 14(5):371–374

Adodo A (2005) New frontiers in African medicine. Metropolitan publishers' Ltd, pp 79–117

Akindele AJ, Adeyemi OO (2006) Analgesic activity of the aqueous leaf extract of *Brysocarpus coccineus*. Niger J health biomed sci 5(1):43–46

Akinyemi KO, Coker AO, Bayagbon C, Oyefolu AOB, Akinsinde K, Omonigbehin EO (2000) Antibacterial screening of five Nigerian medical plants against *S. typhi* and *S. paratyphi*. J Nig Infect Control 3:1–4

Aladesanmi AJ, Iwalewa EO, Adebajo AC, Akinkunmi EO, Taiwo BJ, Olorunmola FO, Lamikanra A (2007) Antimicrobial and antioxidant activities of some nigerian medicinal plants. African J Traditional Complement Altern Med 4(2):173–184

Aliero BL, Umaru MA, Suberu HA, Abubakar A (2001) A handbook of common plants in North Western Nigeria. Usman Danfodiyo University Press, Kano, pp 22–56

Amadi JE, Salami SO, Eze CS (2010) Antifungal properties and phytochemical screening of extracts of African Basil (*Ocimum gratissimum* L.). Agric Biol J North Am 1(2):163–166

Arif T, Bhosalea JD, Kumara N, Mandala TK, Bendreb RS, Lavekara GS, Dabura R (2009) Natural products—antifungal agents derived from plants. J Asian Nat Prod Res 11(7): 621–638

Ayodele SM, Ilondu EM, Onwubolu NC (2009) Antifungal properties of some locally used spices In Nigeria against some rot fungi. Afr J Plant Sci 3(6):139–141
Beck-Sague C, Banerjee S and Jarvis WRJ (1993) Public Health 83:1739
Belhouchet Z, Sautour M, Miyamoto T, Lacaille-Dubois MA (2008) Steroidal saponins from the roots of *Smilax aspera* subsp. *mauritanica*. Chem Pharm Bull (Tokyo) 56:1324
Bergeron C, Marston A, Gauthier R, Hostettmann K (1996) Screening of plants used by North American Indians for antifungal, bactericidal, larvicidal and molluscicidal activities. Int J Pharmacogn 34(4):233–242
Bhadauria S, Kumar P (2011) *In Vitro* antimycotic activity of some medicinal plants against human pathogenic dermatophytes. Indian J Fundam Appl Life Sci 1(2):1–10
Brinker F (1993) Larrea tridentata (D.C.) Coville (Chaparral or Creosote Bush). British J Phytother 3:10
Bryce K (1992) The fifth kingdom. Mycologue Publications, Ontario, p 451
Burkhill HM (1994) Useful plants of west tropical Africa: families E-I, vol 2. Royal Botanical Gardens, Kew, p 85
Burkill HM (1985). Useful plants of west tropical Africa Ed. 2, 1, families A-D, Royal Botanic Gardens, kew
Buss AD, Cox B, Waigh RD (2003) Drug discovery In: Abraham DJ (ed) Burger's medicinal chemistry and drug discovery 6th Edn, vol 1. Wiley, Hoboken, pp 847–900
Butler MS (2005) Natural products to drugs: natural product derived compounds in clinical trials. Nat Prod Rep 22:162–195
Caceres A, Lopez B, Juarez X, Aguila J, Garcia S, Del Aguila J (1993) Plants used in Guatemala for the treatment of dermatophytic infections. J Ethnopharmacol 40:207–213
Céspedes CL, Avila G, Martinez A, Serrato B, Calderon-Mugica JC, Salgado-Garciglia R (2006) Antifungal and antibacterial activities of Mexican tarragon (Tagetes lucida). J Agric Food Chem 54:3521–3527
Como JA, Dismukes WE (1994) Oral azole drugs as systemic antifungal therapy. New Engl J Med 330:263–272
Cowan MM (1999) Plant products as antimicrobial agents. Clin Microbiol Rev 12(4):564–582
Dalziel JM (1955). The useful plants of West Tropical Africa. A crown agent for oversea publication, London, pp 568–570
Darrah HH (1980) The cultivated basils. Buckeye Printing Company, Karachi, pp 112–120
De Leo M, Braca A, De Tomasi N, Norscia I, Morelli I, Battinelli L, Mazzanti G (2004) Phenolic compounds from Baseonema acuminatum leaves: isolation and antimicrobial activity. Planta Med 70:841
Effiong BN, Sanni A (2009) Antifungal Properties and Phytochemical Screening of Crude Extract of *Lemnapauciscostata* (Helgelm) Against Fish Feed Spoilage Fungi. Life Sci J 6(3):19–22
Ensermu K (1994) A revision of Asystasia gangetica (L.) T. Anders. (Acanthaceae). In: Seyani JH, Chikuni AC (eds) Proceedings of the 13th plenary meeting of AETFAT, Zomba, 2–11 April, 1991. Plants of the people, National Herbarium and Botanic Gardens of Malawi, Zomba, 1:333–346
Escalante AM, Santecchia CB, Lopez SN, Gattuso MA, Gutierrez Ravelo A, Delle Monache A, Gonzalez Sierra M, Zacchino SA (2002) Isolation of antifungal saponins from Phytolacca tetramera, an Argentinean species in critic risk. J Ethnopharmacol 82:29
Essiett UA, Bala DN, Agbakahi JA (2010) Pharmacognostic studies of the leaves and stem of Diodia scandens Sw in Nigeria. Scholars Res Libr, Arch Appl Sci Res 2(5):184–198
Etukudo I (2003) *Ethnobotany*: conventional and traditional use of plants, 1st edn. Verdict Press Limited, Uyo 191p
Facchini PJ, Johnson AG, Poupart J, De Luca V (1996) Uncoupled defense gene expression and antimicrobial alkaloid accumulation in elicited opium poppy cell cultures. Plant Physiol 111:687
Farombi EO (2003) African indigenous plants with chemotherapeutic potential and biotechnological approach to the production of bioactive prophylactic agents. Afr J Biotechnol 2:662–671

Farombi EO, Ogundipe OO, Moody JO (2001) Antioxidant and anti-inflammatory activities of *Mallotus oppositifolius* in model systems. Afr J Med Sci 30:213–215
Fleming RV, Walsh TJ, Anaissie EJ (2002) Emerging and less common fungal pathogens. Infect Dis Clin North Am 16:915–933
Galvano F, Piva A, Ritieni A, Galvano G (2001) Dietary strategies to counteract the effect of mycotoxins: a review. J Food Prot 64:120–131
Hamid AA, Aiyelaagbe OO, Ahmed RN, Usman LA, Adebayo SA (2011) Preliminary phytochemistry, antibacterial and antifungal properties of extracts of Asystasia gangetica Linn T. Anderson grown in Nigeria. Adv Appl Sci Res 2(3):219–226
Hamid AA, Aiyelaagbe OO (2011) Preliminary phytochemical, antibacterial and antifungal properties of Alafia barteri stem grown in Nigeria. Eur J Med Plants 1(2):26–32
Hamid AA, Aiyelaagbe OO (2010) The screening of Phytoconstituents, antibacterial and antifungal activities of *Brysocarpus Coccineus* Schum and Thonn. stem (Connaraceae). Adv Environ Biol 4(3):485–489
Harvey AL (2008) Natural products in drug discovery. Drug Discovery Today 13(19/20):894–901
Hassan SW, Bilbis FL, Ladan MJ, Umar RA, Dangoggo SM, Saidu Y, Abubakar MK, Faruk UZ (2006) Evaluation of antifungal activity and phytochemical analysis of leaves, root and stem barks extracts of *Calotropis procera* (Asclepiadaceae). Pak J Biol Sci 9(4):2624–2629
Henley DV, Lipson N, Korah KS, Bioch CA (2007) Pre-pubertal gynecomastia linked to lavender and tea tree oils. N Engl J Med 356(5):479–485
Hostettmann K, Marston A (1994) Search for new antifungal compounds from higher plants. Pure Appl. Chern 66(10/11):2231–223
Hussain HSN, Deeric YY (1991) Plants in Kano ethnomedicine: screening for antimicrobial activity and alkaloids. Int J Pharm 29(1):51–56
Iwata K (1992) Drug resistance in human pathogenic fungi. Eur J Epidemiol 8:407–42
Iwu M (1993) Handbook of African medicinal plants. CRC Press, Boca Raton
Janardhana GR, Raveesha KA, Shetty HS (1998) Modified atmosphere storage to prevent mould-induced nutritional loss in maize. J Sci Food Agric 76:573–578
Lamidi M, Digiorgio C, Delmas F, Favel A, Egele C, Rondi ML, Ollivier E, Uze L, Balansard G (2005) *In vitro* cytotoxic, antileishmanial and antifungal activities of ethnopharmacologically selected Gabonese plants. J Ethnopharmacol 102:185–190
Mahesh B, Satish S (2008) Antimicrobial activity of some medicinal plants against plant and human pathogen. World J Agric Sci 4:839–843
Mann A, Banso A, Clifford LC (2008) An antifungal property of crude plant extracts from *Anogeissus leiocarpus* and *Terminalia avicennioides*. Tanzania J Health Res 10(1):34–38
Mbagwu IG (2001) The effect of long-term storage on the nutrient characteristics of duckweed (*Lemna pauciscostata* Helgelm). J Arid Agri 11:147–149
Mehrabian S, Molabashi Z, Majd A (1995) The antimicrobial effect of garlic (*Allium sativum*) extract on mouth microflora. Iranian J Publ Health 24:39–44
Miller JD (1995) Fungi and mycotoxins in grain: Implications for stored product research. J Stored Prod Res 31:1–16
Morales MR, Simon JE (1996) New basil selection with compact inflorescence for the ornamental market. In: Jamek J (ed.) Progress in new crops. S. press, Alexandria
Nair R, Kalariya T, Chanda S (2005) Antibacterial activity of some selected Indian medicinal flora. Turkish J Biol 29:41–47
Newman DJ, Cragg GM, Snader KM (2000) Natural Prod Rep 17:215–234
Odds FC, Arai T, DiSalvo AF, Evans EGV, Hay RJ, Randhawa HS, Rinaldi MG, Walsh TJ (1992) Nomenclature of fungal diseases. J Med Vet Mycol 30:1
Ofodile LN, Kanife UC, Arojojoye BJ (2010) Antifungal activity of a Nigerian herbal plant. *Chrysanthellum americanum* acta SATECH 3(2):60–63
Ogbebor N, Adekunle AT (2005) Inhibition of conidial germination and mycelial growth of *Corynespora cassiicola* (Berk and Curt) of rubber (*Hevea brasiliensis* muell. Arg.) using extracts of some plants. Afr J Biotechnol 4(9):996–1000

Ogbolu DO, Oni AA, Daini AO, Oloko AP (2007) In vitro antimicrobial properties of coconut oil on *Candida* species in Ibadan. Niger J Med Foods 10(2):173–184
Ogundare AO (2009) The antimicrobial pattern and phytochemical properties of the leaf extract of *Senna podocarpa*. Afr J Microbiol Res 37(7):400–406
Ogundare AO, Adetuyi FC, Akinyosoye FA (2006) Antibacterial activities of *Vernonia tenomeena*. Afr J Biotechnol 5(18):1663–1668
Olli S, Kirti PBJ (2006) Cloning, characterization and antifungal activity of defensin Tfgd1 from *Trigonella foenum-graecum* L. Biochem Mol Biol 39:278
Oliver B (1959) Medicinal plants in Nigeria. Nigerian College of Arts, Science and Technology, Ibadan, p 4
Owoyale JA, Olatunji GA, Oguntoye SO (2005) Antifungal and antibacterial activities of an alcoholic extract of *Senna alata L*eaves. J Appl Sci Environ Manage 9(3):105–107
Onyeyili PA (2000) Anthelminthic efficacy of some plants used in ethnoveterinary practices in the Arid zone of North Eastern Nigeia. RGA No. 28 project Report
Oyelami OA, Onayemi O, Oladimeji FA, Ogundaini AO, Olugbade TA, Onawunmi GO (2003) Clinical evaluation of *Acalypha* ointment in the treatment of superficial fungal skin diseases. Phytother Res 17:555–557
Park Y, Choi BH, Kwak JS, Kang CW, Lim HT, Cheong HS, Hahm KSJ (2005) Kunitz-type serine protease inhibitor from potato (*Solanum tuberosum* L. cv. Jopung). Agric Food Chem 53:6491
Perea S, Thomas F, Patterson TF (2002) Antifungal resistance in pathogenic fungi. Antimicrob Resist, CID 2002:35 (1 Nov), 1073–1080
Pimentel D, Levitan L (1986) Pesticides: amounts applied and amounts reaching pests. Bioscience 36:86–91
Prusti A, Mishra SR, Sahoo S, Mishra SK (2008) Antibacterial activity of some Indian medicinal plants. Ethnobot Leaflets 12:227–230
Rex JH, Rinaldi MG, Pfaller MA (1995) Detection of amphotericin B-resistant Candida isolates in a broth-based system. Antimicrob Agents Chemother 39(4):906–909
Rinaldi MG, Dixon DM (1994) The evolving etiologies of invasive mycoses. Infect Dis Clin Pract 3(suppl):S47
Rispail N, Morris P, Webb KJ (2005) Phenolic compounds: extraction and analysis. In: Marquez AJ (ed) Lotus Japonicus Handbook. pp 349–355
Satish S, Raghavendra MP, Raveesha KA (2009) Antifungal potentiality of some plant extracts against Fusarium sp. Arch Phytopathol Plant Prot 42(7):618–625
Satish S, Raveesha KA, Janardhana GR (1999) Antibacterial activity of plant extracts on phytopathogenic Xanthomonas campestris pathovars. Lett Appl Microbiol 28:145–147
Schultes RE (1978) The kingdom of plants In: Thomson WAR (ed.) Medicines from the Earth. McGraw-Hill Book Co., New York, p 208
Skilicorn P, Spirar W, Journey W (1993) Duckweed aquaculture: a new aquatic farming system for developing countries. A World Bank Publication, p 76. National Agricultural Research Project (NARP), Nigeria, p 309
Siva N, Ganesan S, Banumathy NM (2008) Antifungal effect of leaf extract of some medicinal plants against *Fusarium oxysporum* causing wilt disease of *Solanum melogena* L. Ethnobot Leaflets 12:156–163
Sneader W (1996) Drug prototypes and their exploitation. Wiley, Chichester, pp 525–532
Sofowora AE (1982) Medicinal plants and traditional medicine in Africa, vol 1. Wiley, New York, p 251
Sule WF, Okonko IO, Omo-Ogun S, Nwanze JC, Ojezele MO, Ojezele OJ, Alli JA, Soyemi ET, Olaonipekun TO (2011) Phytochemical properties and in vitro antifungal activity of *Senna alata* Linn. crude stem bark extract. J Med Plants Res 5(2):176–183
Suleiman MN (2010) Fungitoxic activity of neem and pawpaw leaves extracts on *Alternaria Solani*, causal organism of yam rots. Adv Environ Biol 4(2):159–161

Tadeg H, Mohammed E, Asres K, Gebre T (2005) Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders. J Ethnopharmacol 100(1–2):168–175

Turchetti B, Pinelli P, Buzzini P, Romani A, Heimler D, Franconi F, Martini A (2005) *In vitro* antimycotic activity of some plant extracts towards yeast and yeast-like strains. Phytother Res 19(1):44–49

Verastegui MA, Sanchez CA, Heredia NL, Garcia-Alvarado JS (1996) Antimicrobial activity of extracts of three major plants from the Chihuahuan desert. J Ethnopharmacol 52(3):175–177

Wang S, Wu J, Rao P, Ng TB, Ye X (2005) A chitinase with antifungal activity from the mung bean. Protein Expr Purif 40:230

Wang H, Ng TB (2005) An antifungal protein from ginger rhizomes. Biochem Biophys Res Commun 336:100

Wong JH, Ng TB (2005) Vulgarinin, a broad-spectrum antifungal peptide from haricot beans (*Phaseolus vulgaris*). Int J Biochem Cell Biol 37:1626

Yismaw G, Tessema B, Mulu A, Tiruneh M (2008) The *in vitro* assessment of antibacterial effect of papaya seed extract against bacterial pathogens isolated from urine, wound and stool. Ethiop J Med 46(1):71–77

Yoshiaki A, Masao M, Hideyuki I (1999) Acalyphidins M1, M2 a and D1, ellagitannins from *Acalypha hispida*. Phytochemistry 50:667–676

Zhang JD, Cao YB, Xu Z, Sun HH, An MM, Yan L, Chen HS, Gao PH, Wang Y, Jia XM, Jiang YY (2005) In vitro and in vivo antifungal activities of the eight steroid saponins from *Tribulus terrestris* L. with potent activity against fluconazole-resistant fungal pathogens. Biol Pharm Bull 28:2211

Chapter 4
Review of the Antifungal Potential of African Medicinal Plants

Jean Paul Dzoyem and Victor Kuete

Abstract Human fungal infections are increasing due to increased cancer, AIDS, and immunocompromised patients. Herbal drug is an integral part of the treatment of mycosis alongside pharmaceutical antifungal drugs. However, up to 80 % of populations living in Africa rely on medicinal plants for the treatment of diverse affections. Herein, we will discuss the state-of-art in the medicinal plants research in tropical Africa as potential antifungal drugs, the most significant advances as well as the most promising compound so far isolated.

4.1 Introduction

Human fungal infections are increasing due to increased cancer, acquired immune deficiency syndrome (AIDS), and immunocompromised patients (Chian-Yong and Coleman 2011).

The increased use of antifungal agents also resulted in the development of resistance to the drugs. The prevalence of resistance to antifungal agents significantly increased in the past decade (Chian-Yong and Coleman 2011). Resistance to antifungal agents has important implications for morbidity, mortality, and healthcare in the community. *Candida albicans* and *Cryptococcus neoformans* are the most common opportunistic infections in HIV/AIDS patients (Fan-Harvard et al. 1991; Samie et al. 2010), and management of such infections particularly in HIV/AIDS patients is faced with some difficulties, such as resistance to antifungal agents, drug toxicity, and high cost of antifungal agents (Traeder et al. 2008). Up to 90 % of all HIV patients contract fungal infections during the course of the disease, of which 10–20 % die as a direct consequence of fungal infections

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(Hamza et al. 2006; Back-Brito et al. 2009). Therefore, there is a need to search for alternative control methods and the utilization of medicinal plants by the local populations constitutes an important source of new active and renewable antifungal drugs. For centuries, plants have been used by indigenous people to produce medicines that were used to treat different kinds of ailments. Up to 80 % of the African population rely on medicinal plants as antimalarial, antibacterial, and antidiabetic (Schmidt et al. 2008; Samie et al. 2010; Madureira et al. 2012) as well as antifungal remedies (Kuete et al. 2007a, b, c, d, 2008a, b). The present chapter brings out the state-of-art on the antifungal research from medicinal plants in Africa and provides insight of significant results documented up to date.

4.2 Fungal Infections in Africa

Certain fungi have the ability to cause diseases in humans, plants, and animals. Diseases caused by fungi are called mycoses and those affecting humans are classified according to the tissue levels that are colonized. Superficial infections are generally limited to the outer layers of the skin and hair; cutaneous infections are located deeper in the epidermis, hair and nails while subcutaneous infections involve the dermis, and subcutaneous tissues and muscle. In addition, mycotic infections may be systemic: these are invasive infections of the internal organs with the microorganism gaining entry by the lungs, gastrointestinal tract, or through intravenous lines. They may be caused by primary pathogenic fungi or by opportunistic fungi that are of marginal pathogenicity but can infect the immunocompromised host (Vandeputte et al. 2012).

Fungal infections represent a relatively common problem especially in the tropical and subtropical regions of the world, where the warm and humid climates provide a favorable environment for organisms causing superficial mycoses (Shrum et al. 1994). It has been shown that up to 90 % of all immunocompromised patients contract fungal infections at some point during the course of the disease (Diamond 1991) and 10–20 % die as a direct consequence of fungal infection. Dermatophytes and *Candida* species are the most frequent pathogens in humans and animals while *Cryptococcus neoformans* is the fungus that is an important cause of morbidity and mortality in immunocompromised patients (Gumbo et al. 2002; Hiroshi et al. 2008). In Africa, the HIV pandemic has contributed greatly to the emergence of opportunistic fungal pathogens and increased incidence of opportunistic fungal infections over the last two decades (Ampel 1996). The morbidity and mortality impact is especially pronounced in sub-Saharan Africa. Their distribution varies in different countries and in geographical areas and depends on several factors, such as age and host defenses, individual and social behaviors, life style, type of the population, migration of people, and climatic conditions

4.3 Diseases Burden Estimation and Epidemiology Data Collection

Although mycoses are a frequent health problem in African countries, clinical and epidemiological data remain scarce and fragmentary. These mycoses have a significant impact on public health and a devastating impact on the socioeconomic value of Africa low-income rural workers with limited access to public or private health systems. Despite the considerable morbidity and mortality associated with fungal diseases in sub-Saharan Africa, it is recognized that these diseases have been omitted from an expanded list of neglected tropical diseases (Nelesh et al. 2011). The epidemiology of mycoses in Africa has dramatically altered, fungal diseases are not well described, and data regarding incidence are scanty for several reasons. Lack of adequate diagnostic tests makes the identification of the true disease burden resulting fungal diseases difficult. Most African countries are economically disadvantaged and may therefore lack resources for the implementation of infection control practices and diagnostic laboratory services. A majority of the existing mycology laboratories in Africa employ only conventional diagnostic tests such as direct microscopy and cultures. These methods are not sensitive for many important fungal infections, such as invasive candidiasis, where blood culture sensitivity may be as low as 50 %. Data on epidemiology of the mycoses are based on location and activities of trained investigators while other regions go unreported due to lack of personnel. There is a need for systematic collection of global morbidity and mortality data to reveal the dimensions of fungal diseases in Africa. There are some data about the epidemiologic surveys and reports on geographic distribution as well as the burden of the mycotic diseases in some African regions. However, much of this information continues to be either fragmentary or unavailable as fungal diseases are not classified among the notifiable diseases. Consequently, much of the data on their incidence and prevalence, as well as reports on morbidity and mortality are very incomplete or lacking.

During the last three decades, there has been a marked and steady rise in the global incidence of mycotic infections in Africa. The widespread use of broad-spectrum antibiotics, corticosteroids, and invasive surgical procedures has contributed significantly to this increase. Increasingly, fungi that normally occur as saprobes, commensals, or plant pathogens have been recognized as opportunistic pathogens, which possess latent capabilities to cause life-threatening infections in immunodeficient hosts, particularly AIDS patients. In turn, this has increased interest in pathogenic fungi and the diseases they cause. Research on fungal diseases in most institutions is on clinical and epidemiological aspects; some of them concentrate on superficial mycoses while in some others the emphasis is on systemic mycoses. There have been significant contributions on epidemiological and clinico-pathological aspects of several mycoses prevalent in Africa, namely dermatomycoses, mycetoma, sporotrichosis, candidiasis, aspergillosis, cryptococcosis, and histoplasmosis. With the emergence of AIDS, there is greater awareness and interest in studying the prevalence of opportunistic fungal infections in

immunocompromised patients. The publication trends in Africa over the last 10 years showed that most relate to candidiasis and dermatophytosis but publications related to cryptococcosis, histoplasmosis, and zygomycosis are currently on the rise mainly from South Africa.

4.4 Overview of Major Fungal Infections Found in Africa

4.4.1 Dermatophytosis

Dermatophytic fungal infections are among the most commonly diagnosed fungal diseases in Africa. The pathogen spectrum and the clinical manifestations in Africa are totally different from those seen in other continents. The hot and humid environment in Africa is probably the major reason for their high prevalence. Previous epidemiological studies have shown varying prevalence of dermatophytoses in different countries (Shrum et al. 1994; Lohoue et al. 2004; Ada and Tosanwumi 2007; Nweze 2010). In Tunisia, the most common etiologic agent of *Tinea capitis* in adults remains *T. violaceum*. The incidence of *M. canis* seems to increase steeply because of the high frequency of asymptomatic carriage of domestic animals (Mebazaa et al. 2010).

4.4.2 Candidiasis

Candidiasis is a fungal infection caused by any of the *Candida* species. A number of studies have been reported on the prevalence and the epidemiology of different types of candidosis in different countries in Africa. (Blignaut 2007; Enwuru et al. 2008; Pienaar et al. 2010; Nweze and Ogbonnaya 2011; Agwu et al. 2011). *Candida albicans* remains the predominant cause, accounting for over half of all cases, but *Candida glabrata* has emerged as the second most common cause of invasive Candidiasis (Okonkwo and Umeanaeto 2010).

4.4.3 Cryptococcosis

Cryptococcal meningitis is a common and often devastating disease in areas of high HIV prevalence, such as sub-Saharan Africa. Prospective studies from Africa have shown that 10–20 % of deaths among HIV-infected patients are attributable to cryptococcal meningitis. The mortality rate remains unacceptably high, especially in resource-poor areas, with estimation that more than half a million deaths globally per year can be attributed to cryptococcal meningitis (Arthur and Hosseinipour 2010; Kathleen et al. 2011). Researchers have estimated that there

were about one million infections and half a million deaths from HIV-related cryptococcal meningitis worldwide in 2006; sub-Saharan Africa had the highest global burden of cryptococcal meningitis among people living with HIV (Harrison 2009). Studies from Zimbabwe, Rwanda, Central African Republic, Kenya, and Tanzania have all shown increased incidence of cryptococcal meningitis as an AIDS-dependent illness and a leading cause of AIDS mortality (Park et al. 2009).

4.4.4 Blastomycosis

Blastomycosis is a systemic infection caused by the dimorphic fungus *Blastomyces dermatitidis* and most commonly affecting the lungs, skin, and bone. After a number of reports, blastomycosis has now been reported from at least 17 countries in all the major regions of Africa (Baily 1989; Alvarez et al. 2006; Ferchichia et al. 2006; Cheikh et al. 2008). Between 1983 and 1987, 12 new cases were seen in South Africa and Zimbabwe alone; a large proportion of cases has come from these countries in previous years (Carman et al. 1989; Cheikh et al. 2008). Many reported cases, believed to have been acquired in Africa have been diagnosed elsewhere (El Euch et al. 2004; Harket and Baki 2007; Lahmiti et al. 2009); thus the disease appears to be underreported within Africa.

4.4.5 Sporotrichosis

Sporotrichosis is a subcutaneous fungal infection caused by the traumatic implantation of the dimorphic, pathogenic fungus, *Sporothrix schenckii*. It occurs sporadically in many countries but has been particularly common in the mining areas of South Africa (Barros et al. 2011). Sporotrichosis is also known to have a high prevalence in Kwazulu-Natal, but occurs sporadically in the Western Cape. However, the geographical and epidemiological information is still lacking in these and other parts of South Africa (Vismer and Hull 1997). Three cases of sporotrichosis had been encountered in Sudan by 1978, and two in Nigeria (Barros et al. 2011; Motswaledi et al. 2011). It is suggested that the soil in North Africa differs from that in countries with endemic sporotrichosis, and that the plants normally associated with the disease are not found in that part of Africa. The disease is not well documented in the rest of Africa.

4.4.6 Histoplasmosis

Classical histoplasmosis caused by *Histoplasma capsulatum* var. *capsulatum*, and African histoplasmosis caused by *H. capsulatum* var. *duboisii* are both endemic in

Africa. *Histoplasma capsulatum* var. *capsulatum* is known to occur naturally in South Africa, Zimbabwe, and Tanzania (Gugnani 2000; Gumbo et al. 2001; Loulergue et al. 2007). The occurrence of African histoplasmosis associated with HIV infection has been rarely reported although both pathogens coexist in this area (Ndiaye et al. 2011). About 250 cases of the disease have been recorded, nearly 50 % of them have occurred in Nigeria and 25 % of them have been recorded in Niger, Senegal, Congo, Zaire, and Uganda (Sangare et al. 2008; Garcia-Guinon et al. 2009; Ahogo et al. 2009; Eboi et al. 2011). The clinical features and epidemiology of the two forms of the disease in Africa are reviewed (Gugnani 2000; Sanz-Pelaez et al. 2007).

4.5 Plants Used in African Folk Medicine with Documented Antifungal Properties

Several scientific papers have been published by African scientists on the antifungal activities of the medicinal plants used throughout the continent. More than 60 fungal species (Table 4.1) were subjected to the anti-infective studies. Insights of the most representative and most valuable results as collected from scientific databases such as PubMed, Sciencedirect, Scopus, Scirus, Web of Knowledge, and Google Scholar are recorded in Table 4.2. Classification criteria for the activity as previously documented by Kuete (2010) for plant extract [significant activity (MIC $\leq$ 100 μg/ml), moderate (100 $<$ MIC $\leq$ 625 μg/ml), and low or negligible (MIC $>$ 625 μg/ml)] also served as the standard herein. Up to 60 plant families and 160 plant species are reported in the present work (Table 4.2). The most investigated plants families include Acanthaceae, Anacardiaceae, Apocynaceae, Asteraceae, Burseraceae, Combretaceae, Euphorbiaceae, Fabaceae, Guttiferae, Moraceae, Podocarpaceae, Rubiaceae, Rutaceae, and Verbenaceae. The antifungal potential of species from these families will be further discussed.

4.5.1 Acanthaceae

The family Acanthaceae is a taxon of dicotyledonous flowering plants containing almost 250 genera and about 2,500 species. Several plants species of this family are reported for their antifungal activities (Sudhakar et al. 2006; Moshi et al. 2009; Ogunwande et al. 2010; Madhumitha and Saral 2011). Some of the African medicinal plants of the family Acanthaceae reported for their antifungal properties include *Crabbea velutina, Hygrophila auriculata, Thunbergia alata* that showed inhibitory activities against *Candida albicans, Microsporum canis* and *Trichophyton mentagrophytes* (Vlietinck et al. 1995; Deborah et al. 2006).

Table 4.1 Alphabetic list of the fungal species

Species	Abbreviations
Alternaria alternata	*A. alternata*
Alternaria citri	*A. citri*
Alternaria solani	*A. solani*
Aspergillus flavus	*A. flavus*
Aspergillus fumigatus	*A. fumigatus*
Aspergillus niger	*A. niger*
Botrytis cinerea	*B. cinerea*
Candida albicans	*C. albicans*
Candida glabrata	*C. glabrata*
Candida guilliermondii	*C. guilliermondii*
Candida kefyr	*C. kefyr*
Candida krusei	*C. krusei*
Candida lusitaniae	*C. lusitaniae*
Candida parapsilosis	*C. parapsilosis*
Candida pseudotropicalis	*C. pseudotropicalis*
Candida tropicalis	*C. tropicalis*
Candida zeylanoides	*C. zeylanoides*
Cladosporium cladosporioides	*C. cladosporioides*
Cladosporium cucumerinum	*C. cucumerinum*
Cochliobolus heterostrophus	*C. heterostrophus*
Colletotrichum gloeosporioides	*C. gloeosporioides*
Cryptococcus neoformans	*C. neoformans*
Epidermophyton floccosum	*E. floccosum*
Fusarium culmorum	*F. culmorum*
Fusarium oxysporum	*F. oxysporum*
Fusarium solani	*F. solani*
Geotrichum candidum	*G. candidum*
Lasiodiplodia theobromae	*L. theobromae*
Malassezia furfur	*M. furfur*
Microsporum audouinii	*M. audouinii*
Microsporum boulardii	*M. boulardii*
Microsporum canis	*M. canis*
Microsporum gypseum	*M. gypseum*
Microsporum langeronii	*M. langeronii*
Microsporum nanum	*M. nanum*
Penicillium digitatum	*P. digitatum*

(continued)

Table 4.1 (continued)

Species	Abreviations
Phytophthora cryptogea	*P. cryptogea*
Pyrenophora teres	*P. teres*
Pythium ultimum	*P. ultimum*
Rhizoctonia solani	*R. solani*
Rhodotorula rubra	*R. rubra*
Saccharomyces cerevisiae	*S. cerevisiae*
Sclerotium rolfsii	*S. rolfsii*
Scopulariopsis brevicaulis	*S. brevicaulis*
Sporothrix schenckii	*S. schenckii*
Trichoderma viride	*T. viride*
Trichophyton concentrum	*T. concentrum*
Trichophyton longiformis	*T. longiformis*
Trichophyton mentagrophytes	*T. mentagrophytes*
Trichophyton rubrum	*T. rubrum*
Trichophyton soudanense	*T. soudanense*

4.5.2 *Anacardiaceae*

Anacardiaceae is a family of flowering plants bearing fruits that are drupes and in some cases producing urushiol, an irritant (Yi et al. 2008). Anacardiaceae include numerous genera with several of economic importance. They are also known to possess several medicinal checked properties including antifungal potencies (Barra et al. 2007; Rayne and Mazza 2007; Alves et al. 2009; Johann et al. 2010). Antifungal plants of this family as documented by African scientist include *Harpephyllum caffrum, Lannea velutina, Loxostylis alata, Ozoroa insignis, Pseudospondias microcarpa, Rhus vulgaris*, *Schlerocarya birrea, and Protorhus longifolia*. Minimal inhibitory concentration below 100 μg/ml was reported with the acetone extract of *Lannea velutina* against *Aspergillus fumigatus, Microsporum canis, Sporothrix schenkii* (Suleiman et al. 2010), and *Schlerocarya birrea* against *Candida tropicalis* and *C. krusei* (Hamza et al. 2006).

4.5.3 *Apocynaceae*

The Apocynaceae or dogbane family include trees, shrubs, herbs, and lianas. Several plants of this family worldwide were reported for their antifungal properties (Sequeira et al. 2009; Gaitán et al. 2011; Vital and Rivera 2011). Though exceptional activities were not reported from African plants of the family

Table 4.2 African medicinal plants with evidence of their antifungal activities

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
Acanthaceae	*Crabbea velutina* S. Moore	Spirits, Worms (Tabuti et al. 2003)	Tanzania	/	Qlt against *Cal* for Chloroform/ Methanol extract of the whole plant (Deborah et al. 2006)
	Hygrophila auriculata (K. Schum.) Heine	Anthrax, antiseptic, gonorrhea, laryngitis, leprosy, malaria, poliomyelitis, sores (Vlietinck et al. 1995)	Rwanda	β-Sitosterol (Patra et al. 2010)	Qlt against *Cal, Mca, Mme* for ethanol extract from leaves (Vlietinck et al. 1995)
	Thunbergia alata Boj. ex. Sims	Aphta, cholera, diarrhea, impetigo, malaria, otitis, pneumonia, syphilis, yaws (Vlietinck et al. 1995)	Rwanda	caffeoylmalic, feruloylmalic and p-coumaroylmalic acids (Fatima et al. 2002)	Qlt against *Cal, Mca, Mme* for ethanol extract from the whole plant (Vlietinck et al. 1995)
Agavaceae	*Dracaena steudtneri* Engl.	Otitis, scabies, syphilis, yaws (Vlietinck et al. 1995)	Rwanda	/	Qlt against *Cal, Mca, Mme* for ethanol extract from the leaves, roots, fruits extracts (Vlietinck et al. 1995)
Amaryllidaceae	*Cyrtanthus obliquus* (L.f.) Aiton	Venereal diseases (Buwa and Van Staden 2006)	South Africa	Obliquine, 11α-hydroxygalanthamine, 3-epimacronine, narcissidine, tazettine and trisphaeridine (Brine et al. 2002)	MIC > 625 µg/ml against *Cal* for water, ethanol and ethyl acetate bulbs extracts (Buwa and Van Staden 2006)

(continued)

Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
Anacardiaceae	*Harpephyllum caffrum* Bernh.ex Krauss	Gonorrhea (Buwa and Van Staden 2006)	South Africa	Kaempferol 3-*O*-*β*-(2″-sulphatogalactopyranside), quercetin 3-O-*β*-(2″-sulphatogalactopyran oside), 3 -methoxyellagic acid 4-*O*-*β*-galactopyranoside; 3-methoxy gallic acid 5-sodium sulfate, 1, 3-di-*O*-galloyl glucose, 2,3-di-*O*-galloyl g lucose, gallic acid, gentesic acid 2-*O*-glucoside, gentesic acid 5-*O*-glucoside, protocatechuic acid, p-hydroxybenzoic acid, 3-methoxy gallic acid, 3,3′′dimethoxyellagic acid 4-*O*-glucoside, quercetin 3-*O*-arabinopyranoside, kaempferol 3-*O*-rh amnoside, quercetin 3-O-rhamnoside, kaempferol 3-*O*-galactoside, quercetin 3-*O*-galactoside, 3,3′,4-trimethoxyellagic acid, kaempferol, quercetin (Nawwar et al. 2011)	MIC > 625 μg/ml against *Cal* for the stem bark extract (Buwa and Van Staden 2006)
	Lannea velutina A. Rich.	Stomachache, malaria, hepatitis, wound healing, cough and sexually transmitted diseases (Diallo et al. 1991)	Mali	Catechin, epicatechin, tannins (Maiga et al. 2007)	Qlt against *Cal* and *Ccu* for CH_2Cl_2 and roots MeOH extracts (Diallo et al. 2001)
	Loxostylis alata A. Spreng. ex Rchb.	Stimulation of immune system, relief of pain during child birth (Pooley 1993; Pell 2004)	South Africa	/	MIC < 100 μg/ml against *Afu, Mca, Sch* for acetone extract (Suleiman et al. 2010)

(continued)

Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
	Ozoroa insignis Delile	Diarrhea (Tabuti et al. 2003)	Tanzania	6-pentadecylsalicylic acid (He et al. 2002)	Qlt against *Cal* for bark extract (Deborah et al. 2006)
	Pseudospondias microcarpa (A. Rich) Engl.	Chronic cough (Kisangu et al. 2007)	Tanzania	phenolics, tannins, chalcones in the leaves (Kisangu et al. 2007)	Qlt against *Cal* (Kisangu et al. 2007)
	Rhus vulgaris Meikle	Angina, sores (Vlietinck et al. 1995)	Rwanda	/	Qlt against *Cal, Mca, Mme* (Vlietinck et al. 1995); Qlt against *Cal,Tru, Efc, Mca* (Cos et al. 2002)
	Schlerocarya birrea (A. Rich) Hochst.	Fever, stomach ulcers, wounds, infertility, Snake poison (Mabogo 1990)	Zimbabwe, South Africa, Tanzanian	/	Qlt against *Cal* (Adoum et al. 1997) and $100 < \text{MIC} \leq 625$ µg/ml against *Cal, Cgl Cne*, MIC < 100 µg/ml against *Ctr* and *Ckr* (Hamza et al. 2006)
	Protorhus longifolia (Bernh. Ex C. Krauss) Engl.	Diarrhea (Appidi et al. 2008)	South Africa	3-hydroxylanosta-9,24-dien-21-oic acid; 3-·-hydroxylanosta-8,24-dien-21-oic acid (Mosa 2011)	Qlt against *Afu, Cal, Cne, Mca, Sch* (Suleiman et al. 2010) Cytotoxicity of isolated compounds: weak cytotoxic effects on HEK293 (human embryonic kidney) and HEPG2 (hepatocellular carcinoma) cells (Mosa 2011)

(continued)

Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
Annonaceae	*Monodora myristica* Dunal	Venereal diseases (Buwa and Van Staden 2006) and Malaria (Rukunga et al. 2007); intestinal diseases (Noumi and Yomi 2001); scabies, helminthiasis, malaria and dysenteric syndromes (Okpekon et al. 2004); pneumonia, cough, epilepsy and typhoid (Jeruto et al. 2008)	Cameroon	α-phellandrène, *p*-cymène, α-pinène, *β*-myrcène, limonène, cis-pinocarveol, carvacrol (Koudou et al. 2007)	MIC < 10 μg/ml with linear aliphatic alcohols, n-hexacosanol and mixture of fatty acid esters of diunsaturated linear1,2-diols and 10 < MIC ≤ 100 μg/ml with extracts and other compounds against *Cal, Ckr* (Teinkela et al. 2010)
	Xylopia aethiopica	Cough, bronchitis, dysentery, female sterility (Tatsadjieu et al. 2003)	Cameroon	Not identified but the active essential oil from fruits contained *β*-pinene; terpinen-4-ol; sabinene; α-phellandrene; α-terpineol; α- and *trans*-*β*-ocimene (Tatsadjieu et al. 2003)	(fruits essential oils) Qlt on *Cgl* (Tatsadjieu et al. 2003)
Apiaceae	*Centella asiatica* (Linn) Urban	Pharyngitis, dysrmenorrhoea (Pieme et al. 2008)	Cameroon	Ursolic acid lactone, ursolic acid, pomolic acid, 2α,3 α -dihydroxyurs-12-en-28-oic acid, 3-epimaslinic acid, asiatic acid, corosolic acid, 8-acetoxy-1,9-pentadecadiene-4,6-diyn-3-ol, *β* -sitosterol 3-*O*- *β* -glucopyranoside, rosmarinic acid in aerial parts (Yoshida et al. 2005)	Qlt on *Cal, Cke, Gca, Mfu, Afl, Fusp, Psp.* (Pieme et al. 2008) Antiproliferative activity against MK-1, HeLa and B16F10 tumor cell lines (Yoshida et al. 2005)

(continued)

Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
Apocynaceae	*Acokanthera schimperi* (A.DC.) Schweinf.	Headache, epilepsy, amnesia, eye disease, syphilis, rheumatic pain, elephantiasis, scabies, leprosy, Tineacapitis, wound, eczema, warts and swelling (Abebe and Ayehu 1993)	Ethiopia	/	Qlt against *Ani, Cal, Tme* (Hailu et al. 2005)
	Pleioceras barteri Baill	Abortifacient, emmenagogue (Aladesanmi et al. 2007)	Nigeria	/	Qlt on *Cal, Cps, Tru* (Aladesanmi et al. 2007)
	Tabernaemontana crassa Benth	Gonorrhea fungal infections, ovarian trouble, anthrax, headache, constipation, disinfections, homeostasis (Burkill 1985)	Cameroon	dehydrocorydalmine; palmatine; isoursenol; acetate of isoursenol; lupeol (Kuete 2005)	Weak activity for bark methanol extract, with MIC value > 625 µg/ml against *Cal, Ckr* (Kuete 2005)
	Calotropis procera Aiton.	Ringworm, syphilitic sores, toothache, cough and leprosy (Kew 1985), fevers, rheumatism, indigestion, cold, eczema and diarrhea (Kareem et al. 2008)	Nigeria	Procesterol (Khan and Malik 1989)	MIC > 625 µg/ml against *Cal, Afl, Ani, Mbo* (Kareem et al. 2008)
	Crassocephalum multicorymbosum (Klatt) S. Moore	Anthrax, antiseptic, leprosy, sores (Vlietinck et al. 1995)	Rwanda	/	Qlt against *Cal, Mca, Mme* (Vlietinck et al. 1995), Qlt against) *Cal,Tru, Efc, Mca* (Cos et al. 2002
	Xysmalobium undulatum (L.) Ait. f.	Syphilis (Buwa and Van Staden 2006)	South Africa	Pregnenolone (Geuns 1978)	MIC > 625 µg/ml against *Cal* (Buwa and Van Staden 2006)

(continued)

Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
Aspidiaceae	Dryopteris inaequalis (Schlecht.) Kuntze	Worms (Vlietinck et al. 1995)	Rwanda	/	Qlt against *Cal, Mca, Mme* (Vlietinck et al. 1995), Qlt against *Cal,Tru, Efc, Mca* (Cos et al. 2002)
Araliaceae	*Cussonia barteri* Seemann	Stomachache, malaria, hepatitis, wound healing, cough and sexually transmitted diseases (Diallo et al. 1991)	Mali	3-*O*-*β* -*D*-glucopyranosyl betulinic acid 28-*O*-glucopyranosyl (1 → 6)- *β* -*D*-glucopyranosyl ester, Cussosaponin A, B, C, D, E (Harinantenaina et al. 2002)	Qlt against *Cal* and *Ccu* (Diallo et al. 2001)
Asteraceae	*Erigeron floribundus* (Kunth) Sch. Beep.	skin disorders (Kone et al. 2002)	Ivory Coast	/	100 < MIC ≤ 625 µg/ml against *Mgy, Mca, Efl, Mla, Tme, tru, Tso, Sbr.* MIC > 625 µg/ml on *Mca* (Tra Bi et al. 2008)
	Laggera brevipes Oliv. & Hiern	Herpes (Vlietinck et al. 1995)	Rwanda	/	Qlt against *Cal, Mca, Mme* (Vlietinck et al. 1995), Qlt against *Cal,Tru, Efc, Mca* (Cos et al. 2002)
	Solanecio mannii Hook.	Venereal diseases (Buwa and Van Staden 2006) and Malaria (Rukunga et al. 2007); intestinal diseases (Noumi and Yomi 2001); scabies, helminthiasis, malaria and dysenteric syndromes (Okpekon et al. 2004); pneumonia, cough, epilepsy and typhoid (Jeruto et al. 2008)	Cameroon	n-hexacosanol (Teinkela et al. 2010)	MIC < 100 µg/ml with linear aliphatic alcohols, n-hexacosanol and mixture of fatty acid esters of diunsaturated linear1,2-diols and 10 < MIC ≤ 100 µg/ml with extracts and other compounds against *Cal, Ckr* (Teinkela et al. 2010)

(continued)

Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
	Vernonia adoensis (Warp.) SL	Tuberculosis (Kisangu et al. 2007)	Tanzania	Methacrylate, methylbutyrate (Perdue et al. 1993)	Qlt against *Cal* (Kisangu et al. 2007)
	Vernonia aenulans Vatke	Diarrhea, gastroenteritis, gonorrhea, syphilis, yaws (Vlietinck et al. 1995)	Rwanda	/	Qlt against *Cal, Mca, Mme* (Vlietinck et al. 1995), Qlt against *Cal,Tru, Efc, Mca* (Cos et al. 2002)
	Vernonia amygdalina	Diarrhea, gastroenteritis, hepatitis, dysentery, malaria, worms (Vlietinck et al. 1995) Tonic, constipation, fever, high blood pressure, infectious diseases (Iwalokun et al. 2006)	Rwanda Nigeria	Vernolide, Vernodalol (Erasto et al. 2006)	Qlt against *Cal, Mca, Mme* (Vlietinck et al. 1995), Qlt against *Cal,Tru, Efc, Mca* (Cos et al. 2002 Qlt against *Cal* (Okigbo and Mmeka 2008)
	Vernonia lasiopu	Hepatitis (Vlietinck et al. 1995)	Rwanda	/	Qlt against *Cal, Mca, Mme* (Vlietinck et al. 1995)
Bignoniaceae	*Markhamia lutea*	Bronchitis, gonorrhea, leprosy, malaria (Vlietinck et al. 1995)	Rwanda	Verbascoside, isoverbascoside, luteoside A, luteoside B (Kernan et al. 1998)	Qlt against *Cal, Mca, Mme* (Vlietinck et al. 1995), Qlt against *Cal,Tru, Efc, Mca* (Cos et al. 2002)
	Markhamia tomentosa (Benth) K.Schum	Anti snake venom/bite, sore eyes, heart pain, scrotal elephantiasis (Aladesanmi et al. 2007)	Nigeria	/	Qlt on *Cal, Cps, Tru* (Aladesanmi et al. 2007)
	Newbouldia laevis Seem.	Diarrhea, dysentery, worms, malaria, sexually transmitted diseases, dental caries (Eyong et al. 2006)	Cameroon	Newbouldiaquinone A (Eyong et al. 2006); chrysoeriol; newbouldiaquinone; 2-acetylfuro-1,4-naphthoquinone; 2-hydroxy-3-methoxy-9,10-dioxo-9,10-dihydroanthracene-1-carbaldehyde; lapachol; β-sitosterol-3-O-β-dglucopyranoside; oleanolic acid; canthic acid; newbouldiamide; 2-(4-hydroxyphenyl)-Ethyltrioctanoate (Kuete et al. 2007a)	100 < MIC < 625 for bark methanol extract on *Cal, Ckr, Cgl* (Kuete et al. 2007a)

(continued)

Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
	Spathodea campanulata P. Beauv.	Wound healing (Mensah et al. 2003, Houghton et al. 2005; Sy et al. 2005), dyspepsia and peptic ulcer, stomach ulcer, malaria (Ofori-Kwakye et al. 2009)	Ghana	Ursolic acid, tomentosolic acid, 3β,20 β-dihydroxyurs-12-en-28 oic acid. (Amusan et al. 1996)	Qlt against *Cal* (Ofori-Kwakye et al. 2009)
Burseraceae	*Boswellia carteri* Birdw.	Inflammation, wound healing, skin diseases, urinary tract infections, gynecological disorders, respiratory infections, antiseptic, astringent, cicatrisant, rheumatism (Shealy 1998; Stevensen 1998; Wootton 2005)	South Africa	β-Boswellic acid, Acetyl β-boswellic acid, 11-Keto-β-boswellic acid, Acetyl 11-keto- β-boswellic acid, Acetyl 11-methoxy-α -boswellic acid, 9,11-Dehydro-β-boswellic acid, Acetyl 9,11-dehydro-β-boswellic acid, α-Boswellic acid, Acetyl α-boswellic acid, Lupeolic acid, Acetyl lupeolic acid, 12 α-Elemolic acid, Elemonic acid, 3 α -Hydroxytirucalla-7,24-dien-21-oic acid, 3α -Acetoxytirucalla-7,24-dien-21-oic acid, 3 β -Hydroxytirucalla-8,24-dien-21-oic acid, Incensole, Incensole acetate (Banno et al. 2006)	MIC > 625 µg/ml against *Cal* (VanVuuren et al. 2010)
	Boswellia frereana	Inflammation, wound healing, skin diseases, urinary tract infections, gynecological disorders, respiratory infections, antiseptic, astringent, cicatrisant, rheumatism (Shealy 1998; Stevensen 1998; Wootton 2005)	South Africa	α -Pinene, α –Thujene, β-Pinene, Sabinene, Myrcene, Limonene, p-Cymene, α –Copaene, β-Caryo-phyllene, α Humulene (VanVuuren et al. 2010)	MIC > 625 µg/ml against *Cal* (VanVuuren et al. 2010)

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Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
	Boswellia neglecta Birdw	Inflammation, wound healing, skin diseases, urinary tract infections, gynecological disorders, respiratory infections, antiseptic, astringent, cicatrisant, rheumatism (Shealy 1998; Stevensen 1998; Wootton 2005)	South Africa	Canaric acid, α-amyrin, α-amyrone, epi-α-amyrin (Dekebo et al. 2002)	MIC > 625 µg/ml against *Cal* (VanVuuren et al. 2010)
	Boswellia sacra Flueckiger	Inflammation, wound healing, skin diseases, urinary tract infections, gynecological disorders, respiratory infections, antiseptic, astringent, cicatrisant, rheumatism (Shealy 1998; Stevensen 1998; Wootton 2005)	South Africa	α -pinene, α –thujene, β-pinene, sabinene, myrcene, limonene, p-cymene, α –copaene, β-caryo-phyllene, α humulene, δ-cadinene, β-caryophylene oxide (VanVuuren et al. 2010)	MIC > 625 µg/ml against *Cal* (VanVuuren et al. 2010)
	Boswellia thurifera	Inflammation, wound healing, skin diseases, urinary tract infections, gynecological disorders, respiratory infections, antiseptic, astringent, cicatrisant, rheumatism (Shealy 1998; Stevensen 1998; Wootton 2005)	South Africa	α –pinene, β-pinene, limonene, myrcene, β-caryo-phyllene, sabinene, α-humulene, δ-cadinene, β-caryophylene oxide (VanVuuren et al. 2010)	MIC > 625 µg/ml against *Cal* (VanVuuren et al. 2010)

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Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
Cactaceae	*Opuntia lindheimeri* L. Benson	Stomach pains, diarrhea and type II diabetes (Bergaoui et al. 2007)	Tunisia	Tetradecanoic acid, hexadecanoic acid, butyl tetradecanoate, (*E*)-3-butyldiene phthalide (Bergaoui et al. 2007)	Qlt on *Aso, Bci, Fso, Fox, Pul* (Bergaoui et al. 2007)
	Opuntia macrorhiza Engelm	Stomach pains, diarrhea and type II diabetes (Bergaoui et al. 2007)	Tunisia	Butyl tetradecanoate (Bergaoui et al. 2007)	Qlt on *Aso, Bci, Fso, Fox, Pul* (Bergaoui et al. 2007)
	Opuntia microdasys (Lehmann)	Stomach pains, la diarrhea and type II diabetes (Bergaoui et al. 2007)	Tunisia	Hexadecanoic acid, (E)-3-Butyldiene phthalide, butyl tetradecanoate (Bergaoui et al. 2007)	Qlt on *Aso, Bci, Fso, Fox, Pul* (Bergaoui et al. 2007)
Caesalpiniaceae	*Albizia gummifera* (J.F. Gmel.) C.A. Smith	Venereal diseases (Buwa and Van Staden 2006) and almaria (Rukunga et al. 2007); intestinal diseases (Noumi and Yomi 2001); scabies, helminthiasis, malaria and dysenteric syndromes (Okpekon et al. 2004); pneumonia, cough, epilepsy and typhoid (Jeruto et al. 2008)	South Africa and Cameroon	Betulinic acid, monoacylglycerol esters (Teinkela et al. 2010)	MIC > 625 µg/ml against *Cal* (Buwa and Van Staden 2006) MIC < 10 µg/ml with linear aliphatic alcohols, n-hexacosanol and mixture of fatty acid esters of diunsaturated linear1,2-diols and $10 <$ MIC ≤ 100 µg/ml with extracts and other compounds against *Cal, Ckr* (Teinkela et al. 2010)
	Bauhinia galpinii	Diarrhea, infertility (Mabogo 1990)	South Africa	Quercetin-3-O-galactopyranoside, myricetin-3-O-galactopyranoside, 2″-O-rhamnosylvitexin (Aderogba et al. 2007)	$100 <$ MIC ≤ 625 µg/ml against *Cal* and MIC > 625 µg/ml against, *Ckr, Cne* (Samie et al. 2010)

(continued)

Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
	Senna alata (L) Roxb	Hemorrhoids, constipation, inguinal hernia, intestinal parasitosis, gonorrhea, skin infections, syphilis, diabetes, gastroenteritis, jaundice, eczema, thyphoenteritis, ringworm, food poisoning (Pieme et al. 2008), constipation, stomach pains, liver disease, pre-hepatic jaundice (Ssegawa and Kasenene 2007), skin diseases in general (Ajibesin et al.2008), dermatitis (Roosita et al. 2008), skin rash, herpes zoster (Pesewu et al. 2008), infectious diseases (Magassouba et al. 2007), antidiabetic (Abo et al. 2008),	Cameroon	Emodin, kaempferol, aloe-emodin, rhein (Yagi et al. 1998)	Qlt on *Cal, Cke, Gca, Mfu, Afl, Fusp, Psp.* (Pieme et al. 2008) 100 < MIC ≤ 625 µg/ml against *Gca* (Adedayo et al. 1999)
Canellaceae	*Warburgia salutaris* (Bertol. f.) Chiov.	Bark for aphrodisiac, venereal diseases, colds, sore throat, malaria (Mashimbye et al. 1999)	South Africa	Salutarisolide; warburganal; mukaadial; polygodial; isopolygodial (Mashimbye et al. 1999)	MIC > 625 µg/ml against *Cal* (Motsei et al. 2003) MIC > 625 µg/ml against *Cal, Ckr, Cne* (Samie et al. 2010)

(continued)

Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
Capparaceae	*Boscia senegalensis*	Stomachache, malaria, hepatitis, wound healing, cough and sexually transmitted diseases (Diallo et al. 1991)	Mali	/	Qlt against *Cal* and *Ccu* (Diallo et al. 2001)
	Maerua edulis	Fungal infections, wounds, venereal diseases (Samie et al. 2010)	South Africa	/	MIC > 625 µg/ml against *Cal, Ckr, Cne* (Samie et al. 2010)
Capparidaceae	*Buchholzia coriaceae*	Headache, sinusitis and nasal congestion, chest pain, bronchitis, and kidney pains, earache, small pox, fever, fish poison (Dalziel 1937; Kerharo and Bouquet 1950; Bouquet and Debray 1974; Irvine 1961) pot herb in soups (Walker 1953; Ainsle 1937; Watt and Breyer-Brandigk 1962); eye wash (Dalziel 1937; Oliver 1960; Walker and Sillians 1961; Walker 1953)	Nigeria	Lupeol, beta-sitosterol (Ajaiyeoba et al. 2003)	Qlt against *Cal, Pesp, Fox, Afl, Ani* (Ajaiyeoba 2000)
	Capparis tomentosa Lam	Hepatitis, leprosy, malaria, ophthalmia (Vlietinck et al. 1995), lice(Buwa and Van Staden 2006)	Rwanda, South Africa	24-ethylcholestan-5-en-3-ol, N-benzoylphenylalanylaninol acetate (Akoto et al. 2008)	Qlt against *Cal, Mca, Mme* (Vlietinck et al. 1995), Qlt against *Cal,Tru, Efc, Mca* (Cos et al. 2002) MIC > 625 µg/ml against *Cal* (Buwa and Van Staden 2006)

(continued)

Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
	Gynandropsis gynandra	Headache, sinusitis and nasal congestion, chest pain, bronchitis, kidney pains, earache, small pox, fever, fish poison (Dalziel 1937; Kerharo and Bouquet 1950; Bouquet and Debray 1974; Irvine 1961), pot herb in soups (Walker 1953; Ainsle 1937; Watt and Breyer-Brandigk 1962); eye wash (Dalziel 1937; Oliver 1960; Walker and Sillians 1961; Walker 1953)	Nigeria	/	Qlt against *Cal, Pesp, Fox, Afl, Ani* (Ajaiyeoba 2000)
Caricaceae	*Carica papaya* L.	Overcome criminal case, Cough, Promote labor, Sterility, Migraine, Snakebite (Tabuti et al. 2003)	Tanzania	Protocatechuic acid, p-coumaric acid, caffeic acid, kaempferol, quercetin (Canini et al. 2007), butanol, 3-methylbutanol, benzyl alcohol and α-terpineol (Almora et al. 2004)	Qlt against *Cal* (Deborah et al. 2006)
Caesalpinoidiae	*Peltophorum africanum*	Colds, fever, sore throat, sores, ulcers, blisters in the oral cavity, gonorrhea (Mabogo 1990)	South Africa	11-O-(E)-p-coumaroylbergenin, bergenin norbergenin (Mebe and Makuhunga 1992)	MIC > 625 µg/ml against standard ATCC and clinical strains of *Cal* (Steenkamp et al. 2007)
	Schotia brakipetala	Heart disorders, dysentery, diarrhea (Mabogo 1990)	South Africa	Linoleic and oleic acids (Zheng et al. 2005)	MIC > 625 µg/ml against *Cal, Ckr, Cne* (Samie et al. 2010)

(continued)

Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
	Cassia petersiana Bolle (Caesalpinioideae)	Root for aphrodisiac, gonorrhea, syphilis, stomach ache and epilepsy (Samie et al. 2010)	South Africa	Petersone A, stigmasterol-3-O-*β*-glucoside, 5-acetonyl-7-hydroxy-2 hydroxymethylenechromone, 5-acetonyl-7-hydroxy-2 methylchromone. (Djemgou et al. 2007)	MIC > 625 µg/ml against *Cal, Ckr, Cne* (Samie et al. 2010)
Celastraceae	*Cassine transvaalensis*	Piles, venereal diseases, anthhelmintic, laxative, stomach ache, cough, diarrhea, kidney and bladder infections.	South Africa	4′-O-Methylepigallocatechin, (+)-11,11-dimethyl-1,3,8,10tetrahydroxy-9-methoxypeltogynan, canaphylol, canophyllal (Motlhanka et al. 2008)	MIC > 625 µg/ml against *Cal* (Steenkamp et al. 2007) 100 < MIC ≤ 625 µg/ml against *Cal* and MIC > 625 µg/ml against, *Ckr, Cne* (Samie et al. 2010)
Combretaceae	*Anogeissus leiocarpus* (DC.) Guill. et Perr. (L)	Stomach infection, antidiarrhoic, malaria, skin diseases (Fyhrquist et al. 2002)	Togo	*castalagin, flavogallonic acid, ellagic acid (*Shuaibu et al. 2008*)*	100 < MIC ≤ 625 µg/ml against *Cal, Cgu,Cgl, Ckr, Cpa, Ctr, Cze, Gca, Rru, Cne, Tme, Tru, Bci, Mna, Mgy, Aal* and *Ccl* MIC > 625 µg/ml against *Afu,* Tvi and *Sbr,* (Batawila et al. 2005)
	Combretum fragrans F. Hoffm. (L)	Skin diseases, Incurable wounds, hypertension (Fyhrquist et al. 2002), heart problems, worm, wound dressings, mentally ill, scorpion stings (Watt and Breyer- Brandwijk 1962; Hedberg and Hedberg 1982)	Togo, Tanzania	*β*-sitosterol, stigmasterol (Maima et al. 2008)	100 < MIC ≤ 625 µg/ml against *Cze, Gca, Cne, Tme, Bci, Mna, Tru, Ccl.* MIC > 625 µg/ml against *Cal, Cgu,Cgl, Ckr, Cpa, Ctr, Rru, Afu, Tvi, Mgy, Sbr, Aal,* (Batawila et al. 2005) Qlt against *Cal.* (Fyhrquist et al. 2002)

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Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
	Combretum glutinosum Perr. ex DC	Urinary disorders, renal calculi, effective astringent and gargle for ulcerated surfaces, rejuvenative, laxative, nervine, and expectorant, helminthiasis (Adjanohoun et al. 1986, 1989; Dramane and Houngnon 1986)	Benin and Togo	Combreglutinin, 2,3-(S)-hexahydroxydiphenoyl-D-glucose, punicalin, punicalagin (Jossang et al. 1994)	MIC > 625 µg/ml against *Cal, Efl, Mgy, Tme, Tru* (Baba-Moussa et al. 1999)
	Combretum hispidum Laws.	Soft chancre, seminal defects, effective astringent and gargle for ulcerated surfaces, rejuvenative, laxative, nervine, and expectorant, helminthiasis (Adjanohoun et al. 1986 and 1989; Dramane and Houngnon 1986)	Benin and Togo	/	MIC > 625 µg/ml against *Cal, Efl, Mgy, Tme, Tru* (Baba-Moussa et al. 1999)
	Combretum kaiserana F.Hoffm.	Gonorrhea and diarrhea (Fyhrquist et al. 2002) Heart problems, worm, wound dressings, mentally ill, scorpion stings (Watt and Breyer- Brandwijk 1962; Hedberg and Hedberg 1982)	Tanzania	/	Qlt against *Cal*. (Fyhrquist et al. 2002)

(continued)

Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
	Combretum molle G.Don	Fainting, epilepsy, effective astringent and gargle for ulcerated surfaces, laxative, nervine, and expectorant, helminthiasis (Adjanohoun et al. 1986, 1989; Dramane and Houngnon 1986), gonorrhea, Protect against illness (Tabuti et al. 2003); syphilis, influenza, oedema, Skin diseases and wounds (Fyhrquist et al. 2002); Heart problems, worm, wound dressings, mentally ill, scorpion stings (Watt and Breyer- Brandwijk 1962; Hedberg and Hedberg 1982)	Benin, Togo, Tanzania	Ellagitannin, punicalagin, arjunglucoside, sericoside (Asres et al. 2001a, b)	MIC > 625 μg/ml against *Cal* and *Mgy,* 100 < MIC ≤ 625 μg/ml against *Efl, Tme, Tru* (Baba-Moussa et al. 1999) Qlt against *Cal* (Fyhrquist et al. 2002; Deborah et al. 2006)
	Combretum nigricans Lepr	Cephalgia, narcosis, effective astringent and gargle for ulcerated surfaces, rejuvenative, expectorant, helminthiasis (Adjanohoun et al. 1986 and 1989; Dramane and Houngnon 1986)	Benin and Togo	11α-acetoxy-20,24-epoxy-25-hydroxy-dammar-3-one, 20,24-epoxy-11α,25-dihydroxy-dammar-3-one (Simon et al. 2003) arjungenin, arjunglucoside, combregenin, combreglucoside (Jossang et al. 1996)	MIC > 625 μg/ml against *Cal* and *Mgy,* 100 < MIC ≤ 625 μg/ml against *Efl, Tme, Tru* (Baba-Moussa et al. 1999)

(continued)

Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
	Combretum psidioides Welw.	Diarrhea, muscle pain and oedema (Fyhrquist et al. 2002) Heart problems, worm, wound dressings, mentally ill, scorpion stings (Watt and Breyer- Brandwijk 1962; Hedberg and Hedberg 1982)	Tanzania	/	Qlt against *Cal*. (Fyhrquist et al. 2002)
	Combretum sericea Burch.exDC.	Diarrhea, fever, hypertension, bacterial infections (Fyhrquist et al. 2002) Heart problems, worm, wound dressings, mentally ill, scorpion stings (Watt and Breyer- Brandwijk 1962; Hedberg and Hedberg 1982)	Tanzania	/	Qlt against *Cal*. (Fyhrquist et al. 2002)
	Combretum vendae A.E. van Wyk	Leprosy, ophthalmic remedy, and blood purification (Watt and Breyer-Brandwijk 1962)	South Africa	/	MIC < 100 µg/ml against *MCa* for Acetone extract, *Cne* for Hexane extract (Suleiman et al. 2010)
	Combretum zeyheri Sond.	Diarrhea and cancer (Fyhrquist et al. 2002) Heart problems, worm, wound dressings, mentally ill, scorpion stings (Watt and Breyer- Brandwijk 1962; Hedberg and Hedberg 1982).	Tanzania	/	Qlt against *Cal*. (Fyhrquist et al. 2002)

(continued)

Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
	Pteleopsis suberosa Engl. and Diels	Dermatosis, mycosis, jaundice, bronchitis, effective astringent and gargle for ulcerated surfaces, rejuvenative, laxative, nervine, and expectorant, helminthiasis, (Adjanohoun et al. 1986 and 1989; Dramane and Houngnon 1986)	Benin and Togo	Gallocatechin, kaempferol, quercetin, aglycones and triterpenoids saponins (Leo et al. 2006a, b)	100 < MIC ≤ 625 µg/ml against *Efl, Tme, Tru Cal, Mgy,* (Baba-Moussa et al. 1999)
	Terminalia avicennioides Guill. and Perr	Ophthalmic diseases, intermittent fevers, effective astringent and gargle for ulcerated surfaces, nervine, and expectorant, helminthiasis. (Adjanohoun et al. 1986 and 1989; Dramane and Houngnon 1986)	Benin and Togo	Punicalagin, ellagic acid, flavogallonic acid, terchebulin (Shuaibu et al. 2008)	MIC > 625 µg/ml against *Cal* and *Mgy,* 100 < MIC ≤ 625 µg/ml against *Efl, Tme, Tru* (Baba-Moussa et al. 1999)
	Terminalia brachystemma Welw.ex Hiern	Syphilis, toothache, gastric ulcer, venereal diseases, heart diseases, cleans the urinary system, dysentery, gall stones, sore throats, nose-bleeds, and general weakness (Hutchings et al. 1996; Van Wyk et al. 1997; Masoko et al. 2005)	South Africa	Betulinic acid, ursolic acid, catechin, isoorientin, orientin, isovitexin, punicalagin (Liu et al. 2009)	100 < MIC ≤ 625 µg/ml against *Cal, Cne, Afu, Mca, Sch* (Masoko et al. 2005) Punicalagin showed good activity against Ca, Ck, Cp (Liu et al. 2009)
	Terminalia gazensis Bak.f.	Diarrhea, dysmenorrhoea, earache, fattening babies, fever, headache, hook worm, infertility in women (Hutchings et al. 1996; Van Wyk et al. 1997; Masoko et al. 2005)	South Africa	/	100 < MIC ≤ 625 µg/ml against *Cal, Cne, Afu, Mca, Sch* (Masoko et al. 2005)

(continued)

Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
	Terminalia glaucescens Planch. Ex Benth. (L)	Skin diseases (Fyhrquist et al. 2002)	Togo	/	100 < MIC ≤ 625 µg/ml against *Cal, Cgu,Cgl, Ckr, Cpa, Ctr, Cze, Gca, Rru, Cne, Tme, Tru, Mna, Ccl.*MIC > 625 µg/ml against *Afu, Bci, Tvi, Mgy, Sbr, Aal.* (Batawila et al. 2005)
	Terminalia laxiflora (Engl. Et Diels (L)	Asthenia, Skin diseases, dermatitis, Scurfy affection, leprosy and tuberculosis (Fyhrquist et al. 2002)	Togo	/	100 < MIC ≤ 625 µg/ml against *Cal, Cgu,Cgl, Ckr, Ctr, Cze, Gca, Rru, Cne, Tme, Tru, Mna, Aal. Ccl.*MIC > 625 µg/ml against *Cpa, Afu, Bci, Tvi, Mgy, Sbr,* (Batawila et al. 2005)
	Terminalia macroptera Guill. et Perr. (L)	Skin diseases, infectious diseases (Fyhrquist et al. 2002)	Togo	Anillic acid 4-O-β-D-(6′-O-galloyl) glucopyranoside, 3,3′,4′-tri-O-methylellagic acid, 24-deoxy-sericoside, chebuloside II (Conrad et al. 2001)	100 < MIC ≤ 625 µg/ml against *Cal, Cgu,Cgl, Ckr, Cpa, Ctr, Cze, Gca, Rru, Cne, Tme, Tru, Mna, Ccl.*MIC > 625 µg/ml against *Afu, Bci, Tvi, Mgy, Sbr, Aal.* (Batawila et al. 2005)
	Terminalia mollis Laws.	cardiac disorders, filarial, obesity, neuropathy, rejuvenative, laxative, nervine, and expectorant, helminthiasis (Adjanohoun et al. 1986 and 1989; Dramane and Houngnon 1986, Kokwaro 1976)	Benin and Togo	friedelin, catechin, gallocatechin, 3- O - methylellagic acid 4′-O-α – rhamnopyranoside (Liu et al. 2009)	MIC > 625 µg/ml against *Cal* and *Mgy,* 100 < MIC ≤ 625 µg/ml against *Efl, Tme, Tru* (Baba-Moussa et al. 1999) Qlt on *Cal, Ani, Afl, Afu, Cne, Musp* (Mainen et al. 2006)
	Terminalia prunioides M.A. Lawson	Dyphilis, toothache, gastric ulcer, venereal diseases, heart diseases, cleans the urinary system, dysentery, gall stones, sore throats, nose-bleeds and general weakness (Hutchings et al. 1996; Van Wyk et al. 1997; Masoko et al. 2005)	South Africa	/	100 < MIC ≤ 625 µg/ml against *Cal, Cne, Afu, Mca, Sch* (Masoko et al. 2005)

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Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
	Terminalia sambesiaca Engl. & Diels.	Leprosy, pneumonia, scorpion bite, snake bite, swelling caused by mumps (Hutchings et al. 1996; Van Wyk et al. 1997; Masoko et al. 2005)	South Africa	/	100 < MIC ≤ 625 µg/ml against *Cal, Cne, Afu, Mca, Sch* (Masoko et al. 2005)
	Terminalia sericea Burchex	Infected wounds, menorrhage, to dress on magical wounds (Mabogo 1990); Syphilis, toothache, gastric ulcer, venereal diseases, heart diseases, cleans the urinary system, dysentery, gall stones, sore throats, nose-bleeds, general weakness, abdominal disorders, abdominal pains, backache, bilharziasis, chestcoughs, colds, conjunctivitis (Hutchings et al. 1996; Van Wyk et al. 1997; Masoko et al. 2005)	South Africa	Anolignan B (Eldeen et al. 2006)	100 < MIC ≤ 625 µg/ml against *Cal, Cne, Afu, Mca, Sch* (Masoko et al. 2005) MIC > 625 µg/ml against *Cal, Ckr, Cne* (Samie et al. 2010)
Crassulaceae	*Kalanchoe petitiana*	Tapeworm, trachoma, syphilis, different wellings (Abebe and Ayehu 1993)	Ethiopia	24-ethyl-25-dehydrocholesterol, 24-ethyl-Δ0-,Δ5- sterols, 24-ethyl-Δ5, 22E-sterols (Akihisa et al. 1992)	Qlt against *Ani, Cal, Tme* (Hailu et al. 2005)
Cucurbitaceae	*Citrullus colocynthis* Schrad	Rheumatism (Le Flock,1983; Boukef 1986), hypertension Boukef 1986) dermatological problems and gynecological, urinary and pulmonary infections (Le Flock,1983; Boukef 1986; Marzouk et al. 2009)	Tunisia	Isosaponarin, isovitexin, isoorientin 3'-O-methyl ether, 2-O-β-D-glucopyranosylcucurbitacin I, 2-O-β-D-glucopyranosylcucurbitacin L (Delazar et al. 2006)	MIC > 625 µg/ml against *Cal, Cgl, Ckr, Cpa*.(Marzouk et al. 2010)

(continued)

Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
Ebenaceae	*Diospyros abyssinica*	Stomachache, malaria, hepatitis, wound healing, cough and sexually transmitted diseases (Diallo et al. 1991)	Mali	CH_2Cl_2 and MeOH extracts (Diallo et al. 2001)	Qlt against *Cal* and *Ccu* (Diallo et al. 2001)
	Euclea natalensis A. DC.	bronchitis, pleurisy, chronic asthma and urinary tract infection, dye for skin infections caused by *Mycobacterium leprae*, headache and toothache (Palgrave and Drummond,1977)	South Africa	Lupeol, betulin, β-sitosterol, 20(29)-lupene-3-β-isoferulate, shinanolone and octahydroeuclein (Lall et al. 2006)	Qlt against *Physp, Ani, Afl, Ccl* (Lall et al. 2006)
	Diospyros crassiflora Hiern	Whooping cough, leprosy, snake bites, scabies, skin eruptions, dysentery, eye infections, menstrual troubles, abdominal (Bouquet and Debray,1974; Irvine,1961)	Cameroon	Plumbagin, MeOH/CH_2Cl_2 (1:1) extract (Dzoyem et al. 2006 and 2007)	MIC < 25 µg/ml for plumbagin and MIC < 100 µg/ml for extract on *Cal,Cgl, Ckr, Ctr, Cne, Afu, Afl, Ani, Alsp, Clsp, Gca, Fusp, Pesp, Mgy* (Dzoyem et al. 2006 and 2007)
	Diospyros canaliculata De Wildeman	pains, wounds, ulcers, chest pains skin infections (Bouquet and Debray,1974; Irvine,1961)	Cameroon	MeOH extract (Dzoyem et al. 2011)	Qlt; *Mla, Mca, Tso, Tru* (Dzoyem et al. 2006a, 2007) MIC < 25 µg/ml for plumbagin and MIC < 100 mg for extract on *Cke, Cpa, Mfu, Tssp, Rosp, Ssp, Afl, Hesp, Fso* (Dzoyem et al. 2011)

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Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
Euphorbiaceae	*Bridelia mollis*	Anti-emetic, piles, dysentery, burning and itching, wounds (Mabogo 1990)	South Africa	/	MIC > 625 µg/ml against *Cal, Ckr, Cne* (Samie et al. 2010)
	Bridelia micrantha	Burns, infected wounds, tooth ache, eye pains, headaches, fevers (Mabogo 1990)	South Africa	Friedelin, taraxerone, epifriedelinol, taraxerol, gallic acid, ellagic acid, leucodelphinidin, caffeic acid, (Pegel and Rogers 1968)	MIC > 625 µg/ml against *Cal* (Steenkamp et al. 2007) MIC > 625 µg/ml against *Cal, Ckr, Cne* (Samie et al. 2010)
	Clutia abyssinica	Gonorrhea, hepatitis (Vlietinck et al. 1995)	Rwanda	/	Qlt against *Cal, Mca, Mme* (Vlietinck et al. 1995), Qlt against *Cal,Tru, Efc, Mca* (Cos et al. 2002)
	Euphorbia grantii	Gonorrhea, leprosy, poliomyelitis (Vlietinck et al. 1995)	Rwanda	/	Qlt against *Cal, Mca, Mme* (Vlietinck et al. 1995), Qlt against *Cal,Tru, Efc, Mca* (Cos et al. 2002)
	Euphorbia hirta	Aphta, diarrhea, dysentery, gonorrhea, scabies, tinea (Vlietinck et al. 1995) Asthma, respiratory tract inflammations, coughs, chronic bronchitis and other pulmonary disorders, diarrhrea, dysentery, ulcerated oral mucosa, polyhydramnios, chest pain, pneumonia, schizophrenia and hemorrhoids (Pieme et al. 2008)	Rwanda and Cameroon	Afzelin, quercitrin, myricitrin, gallic acid, 3,4-di-O-galloylquinic acid, 2,4,6-tri-*O*-galloyl-D-glucose, 1,2,3,4, 6-penta-*O*-galloyl-*β*-D-glucose (Liu et al. 2007; Chen 1991)	Qlt against *Cal, Mca, Mme* (Vlietinck et al. 1995), Qlt against *Cal,Tru, Efc, Mca* (Cos et al. 2002) Qlt on *Cal, Cke, Gca, Mfu, Afl, Fusp, Psp.* (Pieme et al. 2008)

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Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
	Flueggea virosa (Roxb. ex Wild.) Voigt.	Hydrocele in children, Protect garden (Tabuti et al. 2003)	Tanzania	Flueggines A and B (Zhao et al. 2011)	Qlt against *Cal* (Deborah et al. 2006)
	Jatropha curcas L	Body sores skin infections (Kisangu et al. 2007), abscess in the stomach (Sandberg et al. 2005)	Tanzania	Curcacycline A, complex of 5-hydroxypyrrolidin-2-one and pyrimidine-2,4-dione (Van den Berg et al. 1995; Staubmann et al. 1999)	Qlt against *Cal* (Kisangu et al. 2007)
	Macaranga kilimandscharica	Angina, bilharziosis (Vlietinck et al. 1995)	Rwanda	/	Qlt against *Cal, Mca, Mme* (Vlietinck et al. 1995), Qlt against *Cal,Tru, Efc, Mca* (Cos et al. 2002)
	Pseudolachnostys maprouneifolia	Bark for noisy stomach, venereal diseases, root for pneumonia (Mabogo 1990)	South Africa	/	MIC > 625 μg/ml against *Cal, Ckr, Cne* (Samie et al. 2010)
Fabaceae	*Abrus precatorius* L.	Conjunctivitis, abdominalpain, protectone against danger, gonorrhea, immunity against measles, premature ejaculation (Tabuti et al. 2003)	Tanzania	Abruquinone B, abruquinone G (Limmatvapirat et al. 2004)	Qlt against *Cal* (Deborah et al. 2006)
	Burkea africana	Stomachache, malaria, hepatitis, wound healing, cough and sexually transmitted diseases (Diallo et al. 1991), headache, migraine, dizziness, pain, inflammation and thrush, in addition to utilization as an antineuralgic, wound-healing and toothcleaning agent (Elin et al. 2002; Delaveau et al. 1979)	Mali	3-methyl-5,6-dihydro-beta-carboline (Fiot et al. 2006)	Qlt against *Cal* and *Ccu* (Diallo et al. 2001)

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Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
	Cajanus cajan	Gonorrhea, pneumonia (Vlietinck et al. 1995)	Rwanda	Betulinic acid, biochanin A, cajanol, genistein, 2′-hydroxygenistein, longistylin A, longistylin C, pinostrobin (Duker-Eshun et al. 2004)	Qlt against *Cal, Mca, Mme* (Vlietinck et al. 1995), Qlt against *Cal, Tru, Efc, Mca* (Cos et al. 2002)
	Calpurnia aurea	Amoebic dysentery and diarrhea in animals, killing head lice in humans and ticks in animals, syphilis, diarrhea, leishmaniasis, tapeworm, trachoma, Tinea capitis, wound, scabies, elephantiasis and different wellings (Asres 1986; Abebe and Ayehu 1993; Fullas 2001)	Ethiopia	3β,4α,13α-trihydroxylupanine, calpaurine, lupinine, epilupinine, calpurmenine, 13-hydroxylupanine, calpumine, virgiline, virgiline pyrrolecarboxylic acid ester, calpurmenine pyrrolecarboxylic acid ester (Asres et al. 1986)	Qlt against *Ani, Cal, Tme* (Hailu et al. 2005)
	Crotalaria mesopontica	Anthrax, laryngitis, sores (Vlietinck et al. 1995)	Rwanda	/	Qlt against *Cal, Mca, Mme* (Vlietinck et al. 1995)
	Erythrina abyssinica	Gonorrhea, hepatitis, leprosy, malaria, meningitis (Vlietinck et al. 1995)	Rwanda	5-prenylbutein, 5-deoxyabyssinin II, Abyssinin III, Abyssinone IV, V, Sigmoidin A, B, C, E (Yenesew et al. 2004)	Qlt against *Cal, Mca, Mme* (Vlietinck et al. 1995), Qlt against *Cal,Tru, Efc, Mca* (Cos et al. 2002)
	Glycine javanica	Pneumonia, syphilis (Vlietinck et al. 1995)	Rwanda	/	Qlt against *Cal, Mca, Mme* (Vlietinck et al. 1995), Qlt against *Cal,Tru, Efc, Mca* (Cos et al. 2002)
	Indigofera arrecta	Hepatitis, leprosy, malaria, scabies, sores, worms (Vlietinck et al. 1995)	Rwanda	/	Qlt against *Cal, Mca, Mme* (Vlietinck et al. 1995), Qlt against *Cal,Tru, Efc, Mca* (Cos et al. 2002)

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Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
	Senna didymobotrya	Roots are used to treat sexually transmitted infections (Samie et al. 2010)	South Africa	Emodin, chrysophanol, physcion, knipholone, 10-hydroxy-10-(physcion-7'-yl)-chrysophanol anthrone, 5,10-dihydroxy-2-methyl-9-(physcion-7′-yl)-l,4-anthraquinone (Alemayehu et al. 1996)	MIC > 625 μg/ml against *Cal, Ckr, Cne* (Samie et al. 2010)
Graminae/Poaceae	*Cymbopogon citratus*	Influenza (Tabuti et al. 2003) Malaria (Nadembega et al. 2011)	Nigeria	Geraniol, α-oxobisabolene, citral (Abegaz et al. 1983)	Qlt against *Cal*. (Okigbo and Mmeka 2008)
Gunneraceae	*Gunnera perpensa* Linn.	Gonorrhoea,syphilis, urinary infections (Buwa and Van Staden 2006)	South Africa	MIC > 625 μg/ml against *Cal* (Buwa and Van Staden 2006)	MIC > 625 μg/ml against *Cal* (Buwa and Van Staden 2006)
Guttiferae	*Allanblackia florinbunda*	Cough (Pieme et al. 2008)	Cameroon	/	Qlt on *Cal, Cke, Gca, Mfu, Afl, Fusp, Psp.* (Pieme et al. 2008)
	Garcinia kola Heckel	Masticatory (stimulate the saliva), dysentery, jaundice (Adebisi 2007)	Nigeria	Cycloartenol, 24-methylene-cycloartenol, prenylcouranalactone, kolanone (Madubunyi 1995)	Qlt against *Cal*. (Okigbo and Mmeka 2008)
		Bronchitis and throat nfections (Adesina et al. 1995; Orie and Ekon 1993)			
		Stimulant (Atawodi et al. 1995)			

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Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
		Cough, purgative, anti-parasitic, anti-microbial (Madubunyi 1995, Okunji and Iwu 1991; Adefule-Ositelu et al. 2004) Treatment of diarrhea (Braide 1991) Liver disorders (Iwu et al. 1990) Poison antidote (Kabangu et al.1987) Aphrodisiac (Ajibola and Satake 1992)			
	Garcinia smeathmanii oliver	Bacterial and fungal infections (Kuete et al. 2007b)	Cameroon	Cheffouxanthone; 1,5 dihydroxyxanthone; 1,3,5-trihydroxyxanthone; bangangxanthone A; smeathxanthone B; smeathxanthone A; guttiferone I; isoxanthochymol; friedelin; triacontanyl cafeate (Kuete et al. 2007b)	100 < MIC < 625 for bark extract on *Cal, Cgl, Ckr* (Kuete et al. 2007b)
	Harungana madagascar-iensis (Lam)	Stops menstruation (Hamill et al. 2003) Chronic diarrhea, malaria, jaundice (Kisangu et al. 2007)	Tanzania	Bazouanthrone, feruginin A, harunganin, harunganol A, harunganol B, friedelan-3-one, betulinic acid, astilbin (Moulary et al. 2006; Lenta et al. 2007)	Qlt against *Cal* (Kisangu et al. 2007)

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Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
	Psorospermum febrifugum Spach,	Skin infections (Body sores, skin rushes-Herpes zoster) (Kisangu et al. 2007), Fever,stomach achE,cough (Hamill et al. 2003), diarrhea, Skin rash (Tabuti et al. 2003)	Tanzania	Psorofebrin, 5′-hydroxyisopsorofebrin, vismione D, vismione F, bianthrones A_1, A_{3a} and A_{3b}, (Botta et al. 1985; Abou-Shoer et al. 1993)	Qlt against *Cal* (Kisangu et al. 2007)
	Vismia guineensis (Linn.) Choisy	Malaria, skin diseases, bacterial infections (Mbaveng et al. 2008a)	Cameroon	3-geranyloxy-6-methyl-1, 8-dihydroxyanthraquinone; vismiaquinone; vismiaquinone B; betulinic acid (roots) (Mbaveng et al. 2008a) vismiaquinone; caloxanthone J; *O*1-demethyl-3′, 4′-deoxypsorospermin-3′, 4′-diol; 6-deoxyisojacareubin; 1, 7-dihydroxyxanthone (barks) (Mbaveng et al. 2008a); friedelin; 1, 8-dihydroxy-6-methoxy-3-methylanthraquinone; kaempferol (leaves) (Mbaveng et al. 2008a)	MIC < 100 μg/ml for leaves, bark and roots methanol extract *on Cal, Tme, Tru* (Mbaveng et al. 2008a)
Hyacinthaceae	*Bowiea volubilis* Harv.ex Hook.	Poultice for the treatment of syphilis (Buwa and Van Staden 2006)	South Africa	/	MIC > 625 μg/ml against *Cal* (Buwa and Van Staden 2006)
	Ledebouria ovatifolia (Bak.) Jessop	Veneral, diseases (Buwa and Van Staden 2006)	South Africa	/	MIC > 625 μg/ml against *Cal* (Buwa and Van Staden 2006)

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Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
Hypoxidaceae	*Hypoxis latifolia* Hook.	urinary infections (Buwa and Van Staden 2006)	South Africa	/	MIC > 625 μg/ml against *Cal* (Buwa and Van Staden 2006)
Iridaceae	*Gladiolus dalenii* Van Geel	Meningitis, malaria,diarrhea,ulcers and HIV related fungal infections (Odhiambo et al. 2010; Lukhoba et al. 2006)	Kenya	/	Qlt against *Ani* (Odhiambo et al. 2010)
Irvingiaceae	*Irvingia gabonensis* (Aubry Lecomte ex O'Rorke) Baill.	Gonorrhea, gastro-intestinal and hepatic disorders, wounds infections, diabetes, analgesic (Ngondi et al. 2005)	Cameroon	3-friedelanone; betulinic acid; oleanolic acid; 3,3′,4′-tri-*O*-methylellagic acid; 3,4-di-*O*-methylellagic acid; hardwickiic acid (Kuete and Efferth 2010)	MIC < 100 μg/ml for bark methanol extract on *Cal* (Kuete and Efferth 2010)
Lamiaceae	*Leonotis nepetaefolia* (L.) R. Br.	Anthrax, hepatitis, pneumonia, syphilis, worms (Vlietinck et al. 1995)	Rwanda	10-O-(trans-3,4-dimethoxycinnamoyl)geniposidic acid, 10-O-(p-hydroxybenzoyl)geniposidic acid, acteoside, martynoside, lavandulifolioside (Takeda et al. 1999)	Qlt against *Cal, Mca, Mme* (Vlietinck et al. 1995), Qlt against *Cal,Tru, Efc, Mca* (Cos et al. 2002)
	Plectranthus barbatus Andr.,	Oral thrush, skin rashes, ring worms, Diarrhea (Kisangu et al. 2007), Nausea (Hamill et al. 2003)	Tanzania	Rosmarinic acid, scutellarein 40-methyl ether 7- O-glucuronide, (16S)-coleon E (Fale et al. 2009)	Qlt against *Cal* (Kisangu et al. 2007)
	Tetradenia riparia (Hochst.) Codd	Angina, antiseptic, diarrhea, gastroenteritis, gonorrhea, influenza, malaria, worms, yaws (Vlietinck et al. 1995)	Rwanda	8(14),15-sandaracopimaradiene-7α, 18-diol, ibozol, 7α-hydroxyroyleanone, umuravumbolide, deacetylumuravumbolide, deacetylboronolide, 1′, 2′-dideacetylboronolide (Hakizamungut et al. 1988)	Qlt against *Cal, Mca, Mme* (Vlietinck et al. 1995), Qlt against *Cal, Tru, Efc, Mca* (Cos et al. 2002)

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Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
Leeaceae	*Leea guinieense* Royen ex L.	Spleen in children, edematogenic pelvic inflammatory (Pieme et al. 2008)	Cameroon	/	Qlt on *Cal, Cke, Gca, Mfu, Afl, Fusp, Psp.* (Pieme et al. 2008)
Liliaceae	*Albuca nelsonii* N.E. Br.	Gonorrhea (Buwa and Van Staden 2006)	South Africa	/	MIC > 625 µg/ml against *Cal* (Buwa and Van Staden 2006)
Loganiaceae	*Strychnos decussata*	Sore throat, fever, headache, wounds, vaginal infections (Samie et al. 2010)	South Africa	Akagerine, 17-O-methyl akagerine, 10-hydroxy-21-O-methylkribine, 10-hydroxy-17-O-methylakagerine (Rolfsen et al. 1980)	MIC > 625 µg/ml against *Cal, Ckr, Cne* (Samie et al. 2010)
Loranthaceae	Loranthus micranthus L.	Epilepsy, hypertension, headache, infertility, cancer, menopausal syndrome and rheumatism (Nwude and Ibrahim 1980)	Nigeria	/	MIC > 625 µg/ml against *Ani* and *Cal* with petroleum ether extract (Osadebe and Akabogu 2006)
Lycoperdaceae	*Lycoperdon giganteum* (Pers.)	Sores, abrasion or bruises, deep cut, hemorrhage as well as urinary tract infection (Buswell and Chang 1993)	Nigeria	/	Qlt against (Jonathan and Fasidi 2003)
	Lycoperdon pusilum (Bat. Ex)	sores, abrasion or bruises, deep cut, hemorrhage as well as urinary tract infection (Buswell and Chang 1993; Oso 1977)	Nigeria	/	Qlt against *Cal, Ani, Afl, Mbo and Tco* (Jonathan and Fasidi 2003)

(continued)

Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
Malvaceae	*Gossypium arboretum*	Male contraceptive (Aladesanmi et al. 2007)	Nigeria	/	Qlt on *Cal, Cps, Tru* (Aladesanmi et al. 2007)
	Hibiscus surattensis	Diarrhea, pneumonia (Vlietinck et al. 1995)	Rwanda	/	Qlt against *Cal, Mca, Mme* (Vlietinck et al. 1995), Qlt against *Cal,Tru, Efc, Mca* (Cos et al. 2002)
	Malva parviflora	Asthma, wounds (Abate 1989)	Ethiopia	/	Qlt against *Ani, Cal, Tme* (Hailu et al. 2005)
Menispermaceae	*Albertisia villosa*	Malaria and many infectious diseases (Kambu 1990)	Democratic Republic of Congo	Cycleanine, N-desmethylcycleanine, Cocsoline (Lohombo-Ekomba et al. 2004)	Qlt against *Tlo,Cal, Afl, Mca and Fso* (Lohombo-Ekomba et al. 2004)
	Sphenoceutrum jollyanum Pierre	Chewing sticks, stomachic Aladesanmi et al. 2007)	Nigeria	Columbin, isocolumbin, fibleucin (Moody et al. 2006)	Qlt on *Cal, Cps, Tru* (Aladesanmi et al. 2007)
Melianthaceae	*Bersama lucens* (Hochst.) Szyszyl.	Lice (Buwa and Van Staden 2006)	South Africa	/	MIC > 625 μg/ml against *Cal* (Buwa and Van Staden 2006)
Meliaceae	*Ekebergia senegalensis* A Juss	Antiepileptic, antimalaria Aladesanmi et al. 2007)	Nigeria	Ekeberginine (Saxton 1987)	Qlt on *Cal, Cps, Tru* (Aladesanmi et al. 2007)
	Trichila heudelotti (Oliver) Planch	Sores, heart troubles (Pieme et al. 2008)	Nigeria	/	Qlt on *Cal, Cps, Tru* (Aladesanmi et al. 2007)
Mimosaceae	*Acacia sieberiana* DC. var. woodii (Burtt Davy) Keay & Brenan	Anthrax, diarrhea (Vlietinck et al. 1995)	Rwanda	Dihydroacacipetalin, proacaciberin (Seigler et al. 1975; Nartey et al. 1981)	Qlt against *Cal, Mca, Mme* (Vlietinck et al. 1995), Qlt against *Cal,Tru, Efc, Mca* (Cos et al. 2002)

(continued)

Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
Moraceae	*Dorstenia angusticornis* Engl.	Gastroenteritis, diarrheal infections (Kuete et al. 2007d)	Cameroon	Gancaonin Q; Stipulin; Angusticornin B; Bartericin A (Kuete et al. 2007d)	MIC < 100 μg/ml for twigs methanol extract on *Cal* and *Ckr* (Kuete et al. 2007d)
	Dorstenia barteri Bureau	Snakebite, rheumatiSM, infectious diseases, arthritis (Tsopmo et al. 1999)	Cameroon	Isobavachalcone; stipulin; 4-hydroxylonchocarpin; kanzonol C; amentoflavone (Mbaveng et al. 2008b)	MIC < 100 μg/ml for twigs methanol extract on *Cal, Cgl, Ckr, Mau* and 100 < MIC < 625 μg/ml on *Tru* (Mbaveng et al. 2008b)
	Dorstenia elliptica Bureau	Eyes infections (Bouquet 1969)	Cameroon	Psoralen; O-[3-(2, 2-dimethyl-3-oxo-2H-furan-5-yl)butyl]bergaptol or Dorstenin; Bergapten; O-[3-(2, 2-dimethyl-3-oxo-2H-furan-5-yl)-3-hydroxybutyl]bergaptol; 3-(3,3-dimethylallyl)-4,2',4'-trihydroxychalcone (Kuete et al. 2007e)	MIC < 100 μg/ml for twigs methanol extract on *Cal* and 100 < MIC < 625 μg/ml on *Cgl, Mau, Tru* (Kuete et al. 2007e)
	Dorstenia turbinata Engl.	Gastroenteritis, skin infections, gastro-entérites, infections cutanées, rheumatism (Ngameni et al. 2009)	Cameroon	5-methoxy-3-[3-(β-glucopyranosyloxy)-2-hydroxy-3-methylbutyl]psoralen; 5-methoxy-3-(3-methyl-2,3-dihydroxybutyl)psoralen; (2′S, 3′R)-3′-hydroxymarmesin; 4-hydroxy-3-ethoxybenzaldehyde; 4-methoxyphenol; psoralen; kanzonol C; 4-hydroxylonchocarpin; umbelliferone (Ngameni et al. 2009)	MIC < 100 μg/ml for twigs methanol extract on *Cal, Cgl, Mau, Tru* (Ngameni et al. 2009)
	Ficus cordata Thunb.	Filaris, diarrheal infections and tuberculosis (Kuete et al. 2008a)	Cameroon	β-Amyrin; β-sitosterol-3-*O*-β-d-glucopyranoside; Catechin; Epiafzelechin (Kuete et al. 2008a)	MIC < 100 μg/ml for bark methanol extract on *Cal, Cgl* (Kuete et al. 2008a)

(continued)

Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
	Morus mesozygia Stapf.	Arthritis, rheumatism, malnutrition, debility; pain-killers, stomach disorders, wound infections, gastro-enteritis, peptic ulcer, infectious diseases (Noumi and Dibakto 2002)	Cameroon	Marsformoxide B; moracin Q; moracin T; artocarpesin; cycloartocarpesin; moracin R; moracin S; moracin U; moracin C; moracin M (Kuete et al. 2008a)	MIC < 100 μg/ml for bark methanol extract on *Cal* (Kuete et al. 2008a)
	Treculia acuminata Baillon	Treat skin diseases, dental allergy, amoebic dysentery and AIDS (Kuete et al. 2007f)	Cameroon	Catechin; 6, 9-dihydroxy-megastigmane-3-one; 2,3-ihydroxypropylhexadecanoate (Kuete et al. 2008b)	100 < MIC < 625 μg/ml for twigs methanol extract on *Cal, Cgl, Ckr* (Kuete et al. 2008b)
	Treculia africana Decaisne	Treat skin diseases, dental allergy, amoebic dysentery and AIDS (Bokesch et al. 2004)	Cameroon	Phyllocoumarin; catechin; 6, 9-dihydroxy-megastigmane-3-one (Kuete et al. 2008b)	MIC < 100 μg/ml for leaves methanol extract on *Cal, Ckr* and 100 < MIC < 625 μg/ml on *Cgl* (Kuete et al. 2008b)
	Treculia obovoidea N.E. Brown	Treat skin diseases, dental allergy, amoebic dysentery and AIDS (Bokesch et al. 2004)	Cameroon	Psoralen; bergapten; 7-methoxycoumarin; 7-hydroxycoumarin; 4,2′,4′-trihydroxychalcone; 4,2′,4′-trihydroxy-3-prenylchalcone; 3-hydroxy-4-methoxybenzoic acid; *O*-[3-(2,2-dimethyl-3-oxo-2*H*-furan-5-yl) butyl] bergaptol (Kuete et al. 2008b)	MIC < 100 μg/ml for twigs methanol extract on *Cal, Ckr* and 100 < MIC < 625 μg/ml on *Cgl* (Kuete et al. 2008b)
	Ficus sycomorus L.	Stomach pains, weight in children.	South Africa	Psoralene, *β*-amyrin lupeol, *β*-sitosterol (Abu-Mustafa et al. 1963)	MIC > 625 μg/ml against *Cal* (Steenkamp et al. 2007)

(continued)

Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
	Trilepisium madagascariense	Venereal diseases, arthritis, rheumatism and stomach troubles (diarrhea and dysentery) and also as a pain-killer cutaneous and subcutaneous parasitic infections (Burkill 1985)	Cameroon	Vanillic acid, isoliquiritigenin (Teke et al. 2011)	MIC < 10 µg/ml with isoliquiritigenin and MIC > 625 µg/ml for other samples against *Cal, Ckr, Ctr, Cpa, Clu, Cgl, Cgu, Cne* (Teke et al. 2011)
Molluginaceae	*Glinus oppositifolius* (L.) Aug.DC.	Stomachache, malaria, hepatitis, wound healing, cough and sexually transmitted diseases (Diallo et al. 1991); wound healing; joint pains, inflammations, diarrhea, intestinal parasites, fever, boils and skin disorders ((Diallo et al. 1999; Diallo 2000)	Mali	L-(-)-(N-trans-cinnamoyl)-arginine, kaempferol 3-O-β-D-galactopyranoside, isorhamnetin 3-O-β-D-xylopyranosyl-(1 → 2)-β-D-galactopyranoside, vitexin, vicenin-2,adenosine, L-phenylalanine (Sahakitpichan et al. 2010)	Qlt against *Cal* and *Ccu* (Diallo et al. 2001)
	Limeum pterocarpum	Stomachache, malaria, hepatitis, wound healing, cough and sexually transmitted diseases (Diallo et al. 1991)	Mali	Limeplide, confertifoline, cinnamolide (Ikhiri et al. 1995)	Qlt against *Cal* and *Ccu* (Diallo et al. 2001)
Myrsinaceae	*Maesa lanceolata* Forsskal	Sore throat, tape worms, hepatitis and cholera (Kubo et al. 1987) Dysenteria (Sindambiwe et al. 1996)	Kenya	Maesasaponin I, II, III, IV2, IV3, V 2, V3, VI2, VI 3, and VII 1 (Apers et al. 1999)	Qlt against *Fox, Ani, Pul, Rso, Pcr, Che. Sro, Pte, Phsp* (Okemo et al. 2003)

(continued)

Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
Myrtaceae	*Psidium guajava* Lin.-Holl.	Leaf used as antiseptic, antidiarrhea, Diarrhea,dysentery (Hamill et al. 2003; Aladesanmi et al. 2007)	Nigeria	Morin-3-O-α-L-lyxopyranoside, morin-3-O-α-arbopyranoside, guaijavarin, quercetin (Arima and Danno 2002)	Qlt on *Cal, Cps, Tru* (Aladesanmi et al. 2007)
	Syzyguim guineensis DC	Antitussive, coughs pelvic inflammatory diseases (Pieme et al. 2008)	Cameroon	Betulinic acid, oleanolic acid, 2-hydroxyoleanolic acid, 2-hydroxyursolic acid, arjunolic acid, asiatic acid, terminolic acid, 6-hydroxyasiatic acid, arjunolic acid 28-*β*-glucopyranosyl ester, asiatic acid 28-*β*-glucopyranosyl ester (Djoukeng et al. 2005)	Qlt on *Cal, Cke, Gca, Mfu, Afl, Fusp, Psp.* (Pieme et al. 2008)
Nyctaginaceae	*Boerhavia diffusa* Linn	Diabetes, anti inflammatory, Abscess, Boils (Aladesanmi et al. 2007)	Nigeria	Boeravinone D, boeravinone E, boeravinone G, boeravinone H (Borrelli et al. 2005)	Qlt on *Cal, Cps, Tru* (Aladesanmi et al. 2007)
Ochnaceae	*Ochna natalitia* (Meisn.) Walp.	Headache, and respiratory diseases (Watt and Breyer-Brandwijk 1962)	South Africa	/	MIC < 100 μg/ml against *Mca, Sch* for hexane extract (Suleiman et al. 2010)
	Lophira alata Banks. Ex Gaertn. f.	Toothache, inflammatory diseases and analgesic (Pieme et al. 2008)	Cameroon	lophiroflavans A, B and C, iophirochalcone (Tih et al. 1992)	Qlt on *Cal, Cke, Gca, Mfu, Afl, Fusp, Psp.* (Pieme et al. 2008)
Olacaceae	*Ximenia caffra*	Blood in feces, menorrhage, cough, infertility,venereal diseases, scurvy (Samie et al. 2010)	South Africa	/	MIC > 625 μg/ml against *Cal, Ckr, Cne* (Samie et al. 2010)
Oliniaceae	*Olinia rochetiana*	Eczema, acnea, scabies (Abebe and Ayehu,1993)	Ethiopia	/	Qlt against *Ani, Cal, Tme* (Hailu et al. 2005)

(continued)

Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
Papilionoideae	*Desmodium adscendens*(SW) DC	Dysenteria, abdominal pain, hemorrhoids, urinal infections and gonocorrhea (Pieme et al. 2008)	Cameroon	Dehydrosoyasaponin I (DHS-I), soyasaponin I and soyasaponin III (Rastogi et al. 2011)	Qlt on *Cal, Cke, Gca, Mfu, Afl, Fusp, Psp.* (Pieme et al. 2008)
Phytolaccaceae	*Phytolacca dodecandra*	Ascariasis, gonorrhoea, malaria, rabies, sore throat, rheumatic pain, jaundice, syphilis pruritus, eczema, and viiligo (Abate 1989; Abebe and Ayehu 1993; Fullas 2001)	Ethiopia	Dodecandrin (Ready et al. 1984)	Qlt against *Ani, Cal, Tme* (Hailu et al. 2005)
Piperaceae	*Piper capense*	Wounds, vaginal discharge, infertility, sore throat and tongue sores, infertility (Mabogo 1990)	South Africa	rel-(7S,8S)- Δ8′-3,4,3′,4′-Bis-methylene-dioxy-7.O.2′,8.3′-lignan, Δ8-l′,2′-Dihydro-3,4,3′,4′-bis-methylenedioxy-2′-oxo-8.l′-lignan, Wallichinine, Capentin (Parmar et al. 1997)	100 < MIC ≤ 625 μg/ml against standard ATCC and clinical strains of *Cal* (Steenkamp et al. 2007) MIC > 625 μg/ml against *Cal, Ckr, Cne* (Samie et al. 2010)
Plantaginaceae	*Plantago palmata*	Dysentery, hepatitis, worms (Vlietinck et al. 1995)	Rwanda	Geniposidic acid, epiloganic acid, gardoside, arborescoside, aucubin, verbascoside, plantamajoside (Ronsted et al. 2003)	Qlt against *Cal, Mca, Mme* (Vlietinck et al. 1995), Qlt against *Cal,Tru, Efc, Mca* (Cos et al. 2002)
Plumbaginaceae	*Plumbago zeylanica* Lin.-Holl.	Spirits, appetite stimulant, antiseptic skin disease, scabies, ulcers (Tabuti et al. 2003; Aladesanmi et al. 2007)	Nigeria	/	Qlt on *Cal, Cps, Tru* (Aladesanmi et al. 2007)

(continued)

Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
Podocarpaceae	*Podocarpus elongatus* (Ait.) L' Herit. ex Pers.	Gallsickness (Watt and Breyer-Brandwijk 1962; Hutchings et al. 1996)	South Africa	Phenolic compounds, Condensed tannins, Gallotannins, flavonoids (Abdillahi et al. 2011)	MIC < 100 μg/ml with CH_2Cl_2 extract MIC > 625 μg/ml with other extracts against *Cal* (Abdillahi et al. 2008)
	Podocarpus falcatus (Thunb.) R. Br. ex Mirb.	Stomachache and cattle diseases (Beentje 1994), gonorrhoea and headaches (Pankhurst 2000), Gallsickness (Watt and Breyer-Brandwijk 1962; Hutchings et al. 1996)	South Africa		MIC < 100 μg/ml with CH_2Cl_2 extract MIC > 625 μg/ml with other extracts against *Cal* (Abdillahi et al. 2008)
	Podocarpus henkelii Stapf. ex Dallim. & Jacks.	Gallsickness (Watt and Breyer-Brandwijk 1962; Hutchings et al. 1996)	South Africa		MIC < 100 μg/ml with CH_2Cl_2 extract MIC > 625 μg/ml with other extracts against *Cal* (Abdillahi et al. 2008)
	Podocarpus latifolius (Thunb.) R. Br. ex Mirb.	Stomachache and cattle diseases (Beentje 1994), gallsickness (Watt and Breyer-Brandwijk 1962; Hutchings et al. 1996)	South Africa		MIC < 100 μg/ml with CH_2Cl_2 extract MIC > 625 μg/ml with other extracts against *Cal* (Abdillahi et al. 2008)
Polygonaceae	*Polygonum pulchrum*	Skin infections (Vlietinck et al. 1995)	Rwanda	/	Qlt against *Cal, Mca, Mme* (Vlietinck et al. 1995), Qlt against *Cal,Tru, Efc, Mca* (Cos et al. 2002)

(continued)

Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
Polygalaceae	*Securidaca longepedunculata* Fresen	Bacterial infections (De Tommasi et al. 1993; Arnold and Gulumian 1984), inflammation (De Tommasi et al. 1993), insanity and epilepsy (Mathias 1982). The leaves are used for treating wounds and sores, cough, venereal diseases, snake bite and as a purgative (Chhabra et al. 1991; Hedberg et al. 1983). They are also used to treat tuberculosis (Asres et al. 2001), bilharzia (Kamwendo et al. 1985), rheumatism (Akah and Nwambie 1994; Asres et al. 2001), skin diseases (Odebiyi 1978), headache and mental illness (Msonthi and Magombo 1983), including convulsions in children (Sofowora 1980). The root decoction is used to hasten labor (Kokwaro 1976; Yu 1982), to treat malaria (Chhabra et al. 1991), rheumatism (Kloos et al. 1978), gonorrhea, palpitations, pneumonia, syphilis (Desta 1993), Infusion reduces swellings (Kareru et al. 2008)	Tanzania, South Africa	(4-methoxy-benzo[1,3]dioxol-5-yl)-phenylmethanone, 1,7-dihydroxy-4-methoxyxanthone (2), benzyl 2-hydroxy-6-methoxybenzoate, and methyl 2-hydroxy-6-methoxybenzoate. (Joseph et al. 2006)	Qlt against *Ani, Afu, Cal, Pesp.*(Joseph et al. 2006) Qlt against *Cal* (Deborah et al. 2006) Qlt against *Sce* (Taniguchi et al. 1978) MIC > 625 µg/ml against *Cal, Ckr, Cne* (Samie et al. 2010)

(continued)

Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
Ranunculaceae	*Knowltonia bracteata* Harv. Ex Zahlbar.	Lice (Buwa and Van Staden 2006)	South Africa	/	MIC > 625 µg/ml against *Cal* (Buwa and Van Staden 2006)
Rhamnaceae	*Berchemia discolor*	Infertility (Mabogo 1990), vaginal infections.	South Africa	Nitidulin, amorphigenin, dabinol (Chin et al. 2006)	Qlt against *Cal* (Gundidza and Sibanda 1991) MIC > 625 µg/ml against *Ckr, Cne* and 100 < MIC ≤ 625 µg/ml against *Cal,* (Samie et al. 2010)
	Ziziphus mucronata Willd	Hydrocele, Itchyskin (Tabuti et al. 2003)	Tanzania	Mucronine J, abyssenine A, mucronine D (Auvin et al. 1996)	Qlt against *Cal* (Deborah et al. 2006)
Rosaceae	*Rubus rigidus*	Anthrax, antiseptic, gastroenteritis, gonorrhea, poliomyelitis, sores, syphilis, whooping-cough (Vlietinck et al. 1995)	Rwanda	Cyanidin 3-(6′′-O-a-rhamnopyranosyl-β-glucopyranoside), cyanidin-3-O-β-glucopyranoside (Byamukama et al. 2005)	Qlt against *Cal, Mca, Mme* (Vlietinck et al. 1995), Qlt against *Cal,Tru, Efc, Mca* (Cos et al. 2002)
Rubiaceae	*Massularia acuminata* (G. Don) Bullock	Cure of mouth infections (Aladesanmi et al. 2007)	Nigeria	/	Qlt on *Cal, Cps, Tru* (Aladesanmi et al. 2007)
	Morinda lucida Benth,	Fever, abdominal pain, dysenteria and splenomegaly (Pieme et al. 2008)	Cameroon	Oruwal, oruwalol, damnacanthal, nor-damnacanthal, soranjidiol, alizarin-1-methyl ether, rubiadin, rubiadin-1-methyl ether, 2-methylanthraquinone, anthraquinone-2-aldehyde, 1-hydroxy-2-methylanthraquinone, 1-methoxy-2-methylanthraquinone; hexacosanoic acid (Adesogan 1973)	Qlt on *Cal, Cke, Gca, Mfu, Afl, Fusp, Psp.* (Pieme et al. 2008)

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Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
	Pavetta ternifolia (Oliv.)	Hepatitis, malaria (Vlietinck et al. 1995)	Rwanda	/	Qlt against *Cal, Mca, Mme* (Vlietinck et al. 1995), Qlt against *Cal,Tru, Efc, Mca* (Cos et al. 2002)
	Pavetta crassipes K. Schum	Fever, schistosomiasis, mental illness, convulsions, malaria, hookworms (Amos et al. 1998) and gonorrhea (Katsayal and Abdurahman 2002)	Guinea	Quercetin-3-*O*-rutinoside (Bello et al. 2011)	MIC > 625 μg/ml against *Cal* (Elhadj et al. 2010)
Rutaceae	*Teclea nobilis* Del	Weight loss, Chronic cough (Kisangu et al. 2007), dental caries (Hamill et al. 2003)	Tanzania	Tecleabine, tecleoxine, isotecleoxine, methylnkolbisine, chloro-desnkolbisine, pteleine, isohaplopine-3,3′-dimethylallylether, nobiline, haplopine-3, 3′-dimethylallylether, anhydroevoxine, kokusaginine, 8-methoxy-flindersine, arborinine, 4′,5-dihydroxy-7-prenyloxyflavanone (Al-Rehaily et al. 2003)	Qlt against *Cal* (Kisangu et al. 2007)
	Zanthoxylum capense (Thunb.) Harv.	Syphilis (Buwa and Van Staden 2006)	South Africa	*β*-sitosterol, sitosterol-*β*-D-glucoside, pellitorine, xanthoxylol-*γ*,*γ*-dimethylallyl ether (Steyn et al. 1998)	MIC > 625 μg/ml against *Cal* (Buwa and Van Staden 2006)

(continued)

Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
	Zanthoxylum chalybeum Engl.	Pyomyositis, sterility, uterine fibroids (Tabuti et al. 2003)	Tanzania	/	Qlt against *Cal* (Deborah et al. 2006)
	Zanthoxylum leprieurii Guill. et Perr	stomatitis, gingivitis, bilharzias, antiulcerative, antiseptic urinary, antiseptic, anti-sickler, antimicrobial, antidiarrheic, digestive aid, sialalogue, anti-odontologic, anticancerous, parasticide, laxative (Kerharo and Adam 1974; Sofowora et al. 1975; Noumi 1984; Comoe 1987)	Cameroon	Chelerythrine, trans-α-ocimene, α-terpinolene, 3-δ-carene, α-pinene, p-cymene, sabinene, γ – terpinene, myrcene, limonene, E-β-ocimene, helebelicine A (Ngono et al. 2000; Kuete 2010; Ngoumfo et al. 2010)	Qlt against *Cal, Cne, Mgy, Mme, Tru, Afu, Afl, Sbr and Bci.* (Ngono et al. 2000)
	Zanthoxylum xanthoxyloides Waterm	Scaring, antiseptic, astringent, laxative antiseptic, anti-sickler, digestive aid, parasiticide (Kerharo and Adam 1974; Sofowora et al. 1975; Noumi 1984; Comoe 1987)	Cameroon	A-pinene, trans-β-ocimene, citronellol, sabinene, myrcene, limonene, cytronellyl acetate, α-phellandrene (Kuete 2010)	Qlt against *Cal, Cne, Mgy, Mme, Tru, Afu, Afl, Sbr and Bci.* MIC > 625 μg/ml against *Cal* and *Cne* and 100 < MIC ≤ 625 μg/ml against *Mgy* and *Mme*(Ngono et al. 2000)
Scrophulariaceae	*Verbascum sinaiticum*	Anthrax, post partum diarrhea, hemorrhage, rheumatic pain, elephantiasis, measles, superficial fungal infections and wounds (Abate 1989; Abebe and Ayehu 1993; Fullas 2001)	Ethiopia	Hydrocarpin, sinaiticin, chrysoeriol, luteolin (Afifi et al. 1993)	Qlt against *Ani, Cal, Tme* (Hailu et al. 2005)

(continued)

Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
Simaroubaceae	*Harrisonia abyssinica* Oliv.	Snake bite, fever, hernia (Tabuti et al. 2003)	Tanzania	*β*-sitosterol, stigmasterol, campesterol, *β*-sitostenone, stigmastenone, campestenone, stigmasta-3,5-dien-7-one, stigmasta-3,5,22-trien-7-one, campesta-3,5-dien-7-one (Baldé et al. 2000)	Qlt against *Cal* (Deborah et al. 2006)
Solanaceae	*Solanum incanum*	Gonorrhea, malaria, whooping-cough (Vlietinck et al. 1995)	Rwanda	Stigmasterol-3-*β*-D-glucoside, khasianine, incanumine (Lin et al. 1990)	Qlt against *Cal, Mca, Mme* (Vlietinck et al. 1995), Qlt against *Cal,Tru, Efc, Mca* (Cos et al. 2002)
	Solanum aculeastrum	Jigger wounds, gonorrhea, cancer, particularly Dunal subsp constipation, tubal blockage, gastritis and epilepsy (Pieme et al. 2008)	Cameroon	Solamargine; Solamarine; Solaculine A (Wanyonyi et al. 2002)	Qlt on *Cal, Cke, Gca, Mfu, Afl, Fusp, Psp.* (Pieme et al. 2008)
Tiliaceae	*Glyphae abrevis* (Spreng)	Venereal diseases (Buwa and Van Staden 2006) and Malaria (Muregi et al. 2007; Rukunga et al. 2007); intestinal diseases (Noumi and Yomi 2001); scabies, helminthiasis, malaria and dysenteric syndromes (Okpekon et al. 2004); pneumonia, cough, epilepsy and typhoid (Jeruto et al. 2008)	Cameroon	Hexane, EtOAc extracts *meso*-erythritol, linear aliphatic alcohols, n-hexacosanol, mixture of mono-acylglycerol esters, Mixture of unsaturated linear fatty acid, Mixture of fatty acid esters of diunsaturated linear1, 2-diols (Teinkela et al. 2010)	MIC < 10 μg/ml with linear aliphatic alcohols, n-hexacosanol and mixture of fatty acid esters of diunsaturated linear1, 2-diols and $10 <$ MIC ≤ 100 μg/ml with extracts and other compounds against *Cal, Ckr* (Teinkela et al. 2010)

(continued)

Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
	Triumfetta rhomboidea	Angina, pneumonia, worms (Vlietinck et al. 1995)	Rwanda	Trans-β-caryophyllene, kessane, caryophyllene oxide (Mevy et al. 2006)	Qlt against *Cal, Mca, Mme* (Vlietinck et al. 1995), Qlt against *Cal, Tru, Efc, Mca* (Cos et al. 2002)
Utriciae	*Pouzolzia mixta*	Diarrhea, dysentery (Mabogo 1990)	South Africa	/	MIC > 625 µg/ml against *Cal, Ckr, Cne* (Samie et al. 2010)
Verbenaceae	*Clerodendron myricoides*	Aphta, diarrhea, gonorrhea, hepatitis, laryngitis, leprosy, syphilis, yaws (Vlietinck et al. 1995)	Rwanda	Myricoidine, dihydromyricoidine (Bashwira and Hootele 1988)	Qlt against *Cal, Mca, Mme* (Vlietinck et al. 1995), Qlt against *Cal, Tru, Efc, Mca* (Cos et al. 2002)
	Lantana trifolia	Angina, gonorrhea, hepatitis, leprosy, malaria (Vlietinck et al. 1995), Chronic cough (Kisangu et al. 2007)	Rwanda, Uganda, Tanzania	Scutellarein-7-O -β-D -apiofuranoside, celtidifoline, betonyoside F, verbascoside, vanillic acid, protocatechuic acid, 4-hydroxybenzoic acid, caffeic acid, coumaric acid, martynoside, Scutellarein-7-O-β-D-glucopyranoside, sorbifolin, samioside (Julião et al. 2010)	Qlt against *Cal, Mca, Mme* (Vlietinck et al. 1995), Qlt against *Cal, Tru, Efc, Mca* (Cos et al. 2002) Qlt against *Cal* (Hamill et al. 2003)
	Lippia javanica	Fever, malaria, influenza, measles, lung infections, asthma, chronic coughs and pleurisy, skin disorders stings and bites (Van Wyk et al. 1997)	South Africa	4-ethyl-nonacosane, (E)-2(3)-tagetenone epoxide, myrcenone, piperitenone, apigenin, cirsimaritin, 6-methoxyluteolin 4′-methyl ether (Mujovo et al. 2008)	MIC > 625 µg/ml against *Cal, Ckr, Cne* (Samie et al. 2010)
	Lippia adoensis	Skin diseases including eczema and superficial fungal infections (Abate 1989)	Ethiopia	/	Qlt against *Ani, Cal, Tme* (Hailu et al. 2005)

(continued)

Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
	Lippia rehmannii H. Pearson	Gastrointestinal and respiratory ailments (Linde et al. 2010)	South Africa	Citralborneol, camphor, neryl acetate, isocaryophyllene, p-cymene, *β*-caryophyllene and *β*-caryophyllene oxide (Linde et al. 2010)	Qlt against *Lth, Cgl, Aal, Pdi, Aci, Bci, Fox* (Linde et al. 2010)
Vitaceae	*Rhoicissus tridentata*	Diarrhea, miscarriages (Mabogo 1990). Abdominal pains and swellings, anti-emetics in children, broken bones, cuts, epilepsy, infertility, menorrhagia, during pregnancy to ensure a safe delivery, renal complaints, sprained ankles, stomach ailments, and sores (Hutchings et al. 1996; Van Wyk et al. 1997; Veale et al. 1992)	South Africa, Tanzanian	Eplgallocatechin, gallocatechin, catechin hydrate, mollisacacidin, epicatechin, fisetinidol, epicatechin-3-O-gallate, procyanidin B3, procyanidin B4, Sitosterol (Brookes and Katsoulis 2006)	No activity against *Cal* (Lin et al. 1999) and MIC of 125 μg/ml against *Ckr* (Hamza et al. 2006) MIC > 625 μg/ml against *Cal, Ckr, Cne* (Samie et al. 2010)

Table 4.2 (continued)

Family	Plants species	Traditional used	Area of plant collection	Potentially active compounds	Screened activity
Zygophyllaceae	*Balanites aegyptiaca* (L.) Delile.	Measles (Tabuti et al. 2003)	Tanzania	N-trans-feruloyltyramine, N-cis-feruloyltyramine, vanillic acid, syringic acid, 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone (Sarker et al. 2000)	Qlt against *Cal* (Deborah et al. 2006)
	Peganum harmala L.	Narcotic (Bruneton 2009), abortif (Bellakhdar 1997; Nath et al. 1993)	Egypt	Harmane, Harmine, Harmaline, Harmalol (Gomah 2010), dipegine, dipeginol (Faskhutdinov et al. 2000)	MIC > 625 μg/ml against *Cal* and *Ani* (Gomah 2010) 100 < MIC ≤ 625 μg/ml against *Cal* and *Ani*

Qlt: qualitative analysis based on the diameter of inhibition zone, the percentage of growth inhibition or the bioautographic TLC

Microorganisms: *Alsp: Alternaria sp; Aal: Alternaria alternata; Aci: Alternaria citri; Aso: Alternaria solani; Afl: Aspergillus flavus; Afu: Aspergillus fumigatus; Ani: Aspergillus niger; Bci: Botrytis cinerea; Cal:Candida albicans; Cgl:Candida glabrata; Cgu: Candida guilliermondii; Cke: Candida kefyr; Ckr: Candida krusei; Clu: Candida lusitaniae; Cpa:Candida parapsilosis; Cps: Candida pseudotropicalis; Ctr:Candida tropicalis; cze: Candida zeylanoides; Clsp: Cladosporium; Ccl: Cladosporium cladosporioides; Ccu: Cladosporium cucumerinum; Che: Cochliobolus heterostrophus; Cgl: Colletotrichum gloeosporioides; Cne: Cryptococcus néoformans; Efl: Epidermophyton floccosum; Fcu: Fusarium culmorum; Fox: Fusarium oxysporum; Fso: Fusarium solani; Fusp: Fusarium sp; Gca: Geotrichum candidum; Hsp: Helminthosporum sp; Lth: Lasiodiplodia theobromae; Mfu: Malassezia furfur; Mau: Microsporum audouinii; Mbo: Microsporum boulardii; Mca: Microsporum canis; Mgy: Microsporum gypseum; Mla: Microsporum langeronii; Mme: Microsporum mentagrophytes; Mna: Microsporum nanum; Musp: Mucor sp; Pdi: Penicillium digitatum; Pesp: Pénicillium sp; Phsp: Phoma sp.; Pcr:Phytophthora cryptogea; Physp: Phytophthora sp; Pte: Pyrenophora teres; Pul: Pythium ultimum; Rso: Rhizoctonia solani; Rru: Rhodotorula rubra; Rosp: Rhodotorula sp; Sce: Saccharomyces cerevisiae; Ssp: Scedosporium sp; Sro: Sclerotium rolfsii; Sbr: Scopulariopsis brevicaulis; Sch: Sporothrix schenkii; Tco: Trichophyton concentrum; TTme: Trichophyton mentagrophytes; Tlo:Trichophyton longiformis; Tru: Trichophyton rubrum; Tso: Trichophyton soudanense; Tssp: Trichosporon sp; vi: Trichoderma viride*

Apocynaceae, several of them have been documented for their inhibitory effects against some fungal species. This include *Acokanthera schimperi, Pleioceras barteri*, *Tabernaemontana crassa, Calotropis procera, Crassocephalum multicorymbosum*, and *Xysmalobium undulatum* (Vlietinck et al. 1995; Cos et al. 2002; Hailu et al. 2005; Kuete 2005; Buwa and Van Staden 2006; Aladesanmi et al. 2007; Kareem et al. 2008) (Table 4.2).

4.5.4 Asteraceae

The Asteraceae or Compositae is an exceedingly large and widespread family of vascular plants. Plants of this family are known to have a variety of pharmacological activities. The antifungal potential numbers of them are reported (Tomczykowa et al. 2008; Thembo et al. 2010; Nazaruk et al. 2010; Tabanca et al. 2011; Latha et al. 2011). Some African plants of this family with reported antifungal effects include *Erigeron floribundus, Laggera brevipes*, *Solanecio mannii, Vernonia adoensis, and V. aenulans, V. amygdalina* (Vlietinck et al. 1995; Cos et al. 2002; Kisangu et al. 2007; Okigbo and Mmeka 2008; Tra Bi et al. 2008; Teinkela et al. 2010) (Table 4.2).

4.5.5 Burseraceae

Burseraceae is a moderate-sized family of 17–18 genera and about 540 species of flowering plants. Within this family, several plants of the genus *Boswellia* from South Africa were found to have antifungal properties against *C. albicans* (Van-Vuuren et al. 2010). However, this activity was rather found to be weak with MIC values above 625 μg/ml (Table 4.2).

4.5.6 Combretaceae

Combretaceae is a family of flowering plants in the order Myrtales. The family includes about 600 species of trees, shrubs, and lianas in 18 genera. The family includes the leadwood tree, *Combretum imberbe*. Three genera, *Conocarpus, Laguncularia* and *Lumnitzera*, grow in mangrove habitats (Lumnitzera 2010). Plants of the family Combretaceae are known to possess medicinal purposes. The antifungal potencies of some of them found in all part of the world are documented. This is one of the most investigated plant families in Africa in terms of antifungal activities. Some of the reported antifungal plants of this family include *Anogeissus leiocarpus, Combretum fragrans, C. glutinosum*, *C. hispidum C. kaiserana, C. molle*, *C. nigricans*, *C. psidioides, C. sericea, C. vendae*,

C. zeyheri, *Pteleopsis suberosa*, *Terminalia avicennioides, T. brachystemma*, *T. gazensis, T. glaucescens, T. laxiflora, T. macroptera, T. mollis*, *T. prunioides*, *T. sambesiaca*, and *T.* Burchex (Baba-Moussa et al. 1999; Fyhrquist et al. 2002; Batawila et al. 2005; Masoko et al. 2005; Deborah et al. 2006; Mainen et al. 2006; Liu et al. 2009; Samie et al. 2010) (Table 4.2). Many plants of this family exhibited moderate to good antifungal activities (Table 4.2) on several fungal species. *Combretum vendae* exhibited a significant antifungal activity (MIC $<$ 100 μg/ml) against *M. Canis and C. neoformans* (Suleiman et al. 2010).

4.5.7 Euphorbiaceae

Euphorbiaceae is a large family of flowering plants with 300 genera and around 7,500 species. Most are herbs, but some, especially in the tropics, are also shrubs or trees. Some are succulent and resemble cacti (http://en.wikipedia.org/wiki/Euphorbiaceae). Though good numbers of medicinal plants of the family Euphorbiaceae have been studied in Africa (Table 4.2), few researchers emphasized on the extent of their antifungal activities. However, qualitative evaluation revealed the inhibitory activity of *Clutia abyssinica, Euphorbia grantii*, *E. hirta, Macaranga kilimandscharica* against *C. albicans, M. canis, M. mentagrophytes, T. rubrum,* and *Epidermophyton floccosum* (Vlietinck et al. 1995; Cos et al. 2002), *Flueggea virosa, Jatropha curcas*, and *Abrus precatorius* against *C. albicans* (Deborah et al. 2006; Kisangu et al. 2007).

4.5.8 Fabaceae

The Fabaceae or Leguminosae, commonly known as the legume, pea, or bean family, is a large and economically important family of flowering plants. The group is the third largest land plant family, behind the Orchidaceae and Asteraceae, with 730 genera and over 19,400 species (Stevens 2001). Several plants of these families are known to have a variety of medicinal purposes including antifungal effects (Lopes et al. 2011; Mikaeili et al. 2011). Qualitative activities were reported for *Abrus precatorius* against *C. albicans* (Deborah et al. 2006), *Burkea africana* against *C. albicans* and *C. cucumerinum* (Diallo et al. 2001), *Cajanus cajan* against *C. albicans, M. canis, Microsporum mentagrophytes* (Vlietinck et al. 1995), *Erythrina abyssinica, Glycine javanica*, *Indigofera arrecta* against *C. albicans, M. Canis,* and *M. mentagrophytes* (Vlietinck et al. 1995).

4.5.9 Guttiferae

The Clusiaceae or Guttiferae is a family of plants formerly including about 37 genera and 1,610 species of trees and shrubs, often with milky sap and fruits or capsules for seeds (Gustafsson et al. 2002). Some of the reported antifungal plants of this family from Africa include *Allanblackia florinbunda, Garcinia kola, Garcinia smeathmanii, Harungana madagascariensis*, *Psorospermum febrifugum*, and *Vismia guineensis* (Kisangu et al. 2007; Kuete et al. 2007b; Mbaveng et al. 2008a; Pieme et al. 2008; Okigbo and Mmeka 2008). Significant activity with MIC values below 100 μg/ml was reported with leaves, bark, and roots methanol extract of *V. guineensis on C. albicans, T. mentagrophytes, T. rubrum* (Mbaveng et al. 2008a) meanwhile *G. smeathmanii* exhibited a moderate activity against *C. albicans, C. glabrata, C. krusei* (Kuete et al. 2007b).

4.5.10 Moraceae

Moraceae, often called the mulberry family or fig family, are a family of flowering plants comprising about 40 genera and over 1,000 species. Several genus of this family were reported for their antifungal properties, the most represented being *Dorstenia, Ficus, Morus, Artocarpus* etc. (Aref et al. 2010; Jagtap and Bapat 2010; Mokoka et al. 2010; Kuete et al. 2007f, 2011). Several plant extracts from the genus *Dorstenia, Ficus, Morus,* and *Treculia* significantly (MIC values below 100 μg/ml) inhibited the growth of many fungi such as *C. albicans, C. glabrata, C. Krusei and M. audouinii* (Table 4.2).

4.5.11 Other Most Investigated Plant Families: Podocarpaceae, Rubiaceae, Rutaceae, and Verbenaceae

Podocarpus is the well-investigated genus of the family Podocarpaceae, and several taxons such as *P. elongatus, P. falcatus, P. henkelii,* and *P. latifolius* showed significant antifungal activity against *C. albicans* (Abdillahi et al. 2008) (Table 4.2). Within the family Rubiaceae, plants such as *Massularia acuminate*, *Morinda lucida*, and *Pavetta* spp were also reported to have antifungal activities against many fungal species (Cos et al. 2002; Aladesanmi et al. 2007; Pieme et al. 2008), though the reported data were rather qualitative. Some plants of the families Rutaceae and Verbenaceae were also reported for their antifungal activities. However, the effects published were either qualitative, or moderately active (Table 4.2).

4.6 Antifungal Natural Products of African Medicinal Plants

Plants are rich sources of bioactive secondary metabolites of wide variety such as tannins, terpenoids, saponins, alkaloids, flavonoids, and other compounds, reported to have in vitro antifungal properties (Arif et al. 2009). Since the plant kingdom provides a useful source of lead compounds of novel structure, a wide-scale investigation of species from the tropics has been considered. Therefore, the research on natural products and compounds derived from natural products has accelerated in recent years due to their importance in drug discovery (Arif et al. 2009). A series of molecules with antifungal activity against different strains of fungus have been found in plants, which are of great importance to humans. Numbers of scientific data were published providing the scientific evidence of African medicinal plants, most of them dealing with crude extracts. However, some African scientists put more emphasis on phytochemical studies of antimicrobial extracts and documented some compounds, belonging mostly to two main groups of secondary metabolites, namely phenolics and alkaloids for their antifungal activities. Compounds belonging to terpenoids rather showed poor antifungal inhibitory effects. In the present review, the classification criteria for the activity that will as previously documented follows: significant activity (MIC < 10 μg/ml), moderate ($10 <$ MIC ≤ 100 μg/ml), and low or negligible (MIC > 100 μg/ml) (Kuete 2010; Kuete and Efferth 2010).

4.6.1 Terpenoids

Terpenoids are the largest and most widespread class of secondary metabolites, mainly in plants and lower invertebrates. A few of them have been used for therapeutic purposes for centuries; but in recent decades the level of research activity in isolating and studying new terpenoids has shown no sign of abation (De las Heras et al. 2003). Generally, terpenoids have low antimicrobial potentials, compared to phenolic compounds. Nevertheless, some of the terpenoids such as the cymbopogonol and citral showed antifungal activity against *C. albicans* (Ragasa et al. 2008). Also, the hardwiickic acid, isolated from *Irvingia gabonensis* presented moderate activity on many other bacterial species and *Candida* spp (Kuete et al. 2007c).

4.6.2 Phenolic Compounds

Flavonoids isolated from African medicinal plants have been reported for their antimicrobial activities (Fig. 4.1). Amongst them are chalcones, flavones, and

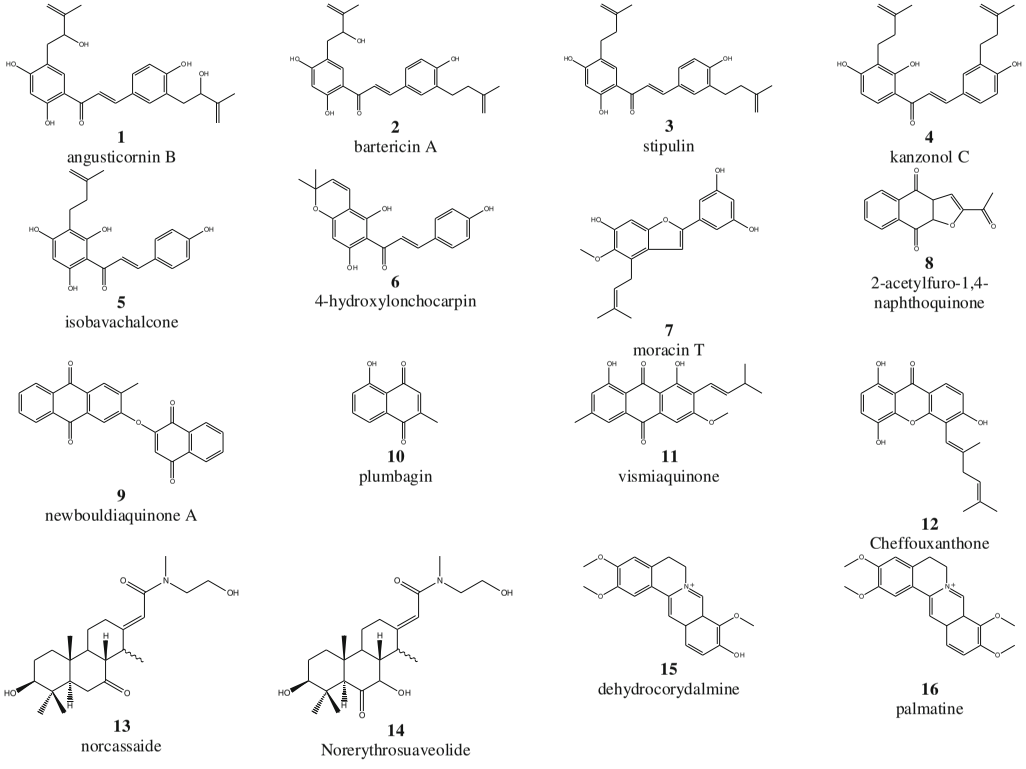

Fig. 4.1 Chemical structures of some antifungal compounds isolated from African medicinal plants

isoflavones. Prenylated chalcones compounds such as angusticornin B (**1**) and bartericin A (**2**), stipulin (**3**) were reported to be very active against *C. albicans, C. glabrata,* and *C. krusei* (Kuete et al. 2007d). Mbaveng et al. (2008b) also demonstrated the antifungal activity of kanzonol C (**4**), isobavachalcone (**5**), 4-hydroxylonchocarpin (**6**). MIC below 2 μg/ml was reported for compound **2** against *C. albicans, C. glabrata,* and *C. krusei* (Kuete et al. 2007d). Aryl-benzofurans (Fig. 4.1) were isolated from *Morus mesozygia*, moracin T (**7**) was very active (MIC of 10 μg/ml) against *C. albicans* (Kuete et al. 2009).

Naphthoquinones isolated from African plants were reported for their activities against fungi (Fig. 4.1). MIC < 10 μg/mL were documented for many of them including 2-acetylfuro-1,4-naphthoquinone (**8**) against *C. albicans* and *C. glabrata* (Kuete and Efferth 2010). Several other quinones (Fig. 4.1) also demonstrated significant antifungal activities namely newbouldiaquinone A (**9**), plumbagin (**10**), and vismiaquinone (**11**) (Dzoyem et al. 2006a, 2007; Kuete et al. 2007a, g). MIC values below 25 μg/ml for plumbagin were reported on *C. albicans, C. glabrata, C. krusei, C. tropicalis, Cochliobolus heterostrophus, Aspergillus flavus, A. niger, Geotrichum candidum, and Microsporum gypseum* (Dzoyem et al. 2006a, b and 2007). A xanthones (Fig. 4.1) isolated from *Garcinia smeathmanii* (Kuete et al. 2007b) named cheffouxanthone (**12**) showed good activity against *C. albicans, C. glabrata* and *C. krusei* (Kuete et al. 2007b).

4.6.3 Alkaloids

Compared with phenolics, very few antifungal alkaloids have been isolated so far from African medicinal plants. This is due to the fact that few numbers of plants families contain this class of compounds (Bruneton 1999). Four of the most active antifungal alkaloids reported so far from the medicinal plants of Africa include norcassaide (**13**) and norerythrosuaveolide (**14**) isolated from *Erythrophleum suaveolens* and reported to have significant inhibitory (MIC < 10 μg/mL) activities against *C. albicans* and *C. krusei* (Ngounou et al. 2005) as well as dehydrocorydalmine (**15**) and palmatine (**16**) from *Tabernaemontana crassa* with significant effects *C. krusei* (Kuete 2005).

4.7 Conclusions

The present chapter brings some insight on the state-of-art in the potential of medicinal plant research in Africa as antifungal agent, on the basis of published works available in Pubmed, Sciendirect, Scopus, Web of Knowledge, Scirus, and so on. It highlights the efforts made by researchers from African countries. However, the study of the mechanism of action is still at a very preliminary phase. This gap is to be taken as a challenge for the near future.

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References

Abate G, Demissew S (eds) (1989) Ethiopian traditional medicine. Addis Ababa University Press, Addis Ababa, pp 99–183

Abdillahi HS, Finnie JF, Van Staden J (2011) Anti-inflammatory, antioxidant, anti-tyrosinase and phenolic contents of four *Podocarpus* species used in traditional medicine in South Africa. J Ethnopharmacol 136:496–503

Abdillahi HS, Stafford GI, Finnie JF, VanStaden J (2008) Antimicrobial activity of South African *Podocarpus* species. J Ethnopharmacol 119:191–194

Abebe D, Ayehu A (1993) Medicinal plants and enigmatic health practices of Northern Ethiopia. Birhanna Selam Printing Press, Addis Ababa

Abegaz B, Yohannes GP, Dieter KR (1983) Constituents of the essential oil of ethiopian *Cymbopogon citratus* stapf. J Nat Prod 46(3):424–426

Abo KA, Fred-Jaiyesimi AA, Jaiyesimi AEA (2008) Ethnobotanical studies of medicinal plants used in the management of diabetes mellitus in South Western Nigeria. J Ethnopharmacol 115:67–71

Abou-Shoer M, Suwanborirux K, Habib MAA, Chang CJ, Cassady MJ (1993) Xanthones and vismiones from *Psorospermum febrifugum*. Phytochemistry 34(5):1413–1420

Abu-Mustafa EA, El-Tawil BAH, Fayez MBE (1963) Constituents of local plants-IV.: *Ficus carica* L., *F. sycomorus* L. and *F. salicifolia* L. leaves. Phytochemistry 3(6):701–703

Ada CN, Tosanwumi VO (2007) Epidemiology of dermatophytoses in a rural community in Eastern Nigeria and review of literature from Africa. Mycopathologia 164:149–158

Adebisi AA (2007) Bitter cola: the African wonder nut. http://www.Cifor.Cgiar.org/publications/pdf-files/books/Restution-Africa-case/NTFP-Africa-case-part2.pdf

Adedayo O, Anderson WA, Moo-Young M, Kolawole DO (1999) Antifungal properties of some components of *Senna Alata* flower. Pharmaceut biol 37:369–374

Adefule Ositelu AO, Adefule AK, Oosa BO, Onyenefa PC (2004) Antifungal activity of *Garcinia kola* nut extract as an ocular bacterial isolates in lagos. Nig Qt J Hosp Med 14:112–114

Aderogba MA, Mcgaw LJ, Ogundaini AO, Eloff JN (2007) Antioxidant activity and cytotoxicity study of the flavonol glycosides from *Bauhinia galpinii*. Nat Prod Res 21(7):591–599

Adesina SK, Gbile ZO, Odukoya OA, Akinwusi DD, Illoh HC and Jayeola AA (1995) Survey of indigenous useful plants of West Africa with special emphasis on medicinal plants and issues associated with their management. The United Nations University programme on natural resources in Africa; 2nd edn. pp, 84–85

Adesogan EK (1973) Anthraquinones and anthraquinols from *Morinda lucida*: the biogenetic significance of oruwal and oruwalol. Tetrahedron 29(24):4099–4102

Adjanohoun EJ, Adjakidje V, Ahyi MRA, Ake AL, Akoegninou A, Dossa CZ, Gassita JN, Gbaguidi N, Goudote E, Guinko S, Houngnon P, Lo I, Keita A, Kiniffo HV, Kone-Bamba D, Nseyya AM, Saadou M, Sodogandji R, de Souza S, Tchabi A and Zohoun T (1989) Contribution aux Etudes Ethnobotaniques et Floristiques du Benin. ACCT, Paris, pp 184–199

Adjanohoun EJ, Ahyi MRA, Ake Assi L, Akpagana K, Chibon P, El Hadj WA, Eyme J, Garba M, Gassita JN, Gbeassor M, Goudote E, Guinko S, Hodouto K–K, Houngnon P, Keita A, Keoula Y, Kluga OWP, Lo I, Siamevi KM, Taffame K (1986) Contribution aux Etudes Ethnobotaniques et Floristiques du Togo. ACCT, Paris

Adoum OA, Dabo NT, Fatope MO (1997) Bioactivities of some savanna plants in the brine shrimp lethality test and in vitro antimicrobial assay. Int J Pharmacogn 35:334–337

Afifi SAM, Ahmed MM, Pezzuto MJ, Kinghornt AD (1993) Cytotoxic flavonolignans and flavones from *Verbascum sinaiticum* leaves. Phytochemistry 34(3):839–841

Agwu E, Ihongbe JC, McManus BA, Moran GP, Coleman DC, Sullivan DJ (2011) Distribution of yeast species associated with oral lesions in HIV-infected patients in Southwest Uganda. Med Mycol. doi:10.3109/13693786.2011.604862

Ahogo KC, Sangare A, Gbery IP, Ecra E, Kaloga M, Kassi K, Kouame K, Kourouma AK, Abadjinan A, Kacou DE and Kanga JM (2009) Cutaneous histoplasmosis due to *Histoplasma capsulatum* variety *duboisii* in an immune competent child. About one case in Abidjan, Côte d'Ivoire. Bulletin de la Société de Pathologie Exotique 102(3):147–149

Ainsle JR (1937) List of plants used in native medicine in Nigeria. Oxford University Press, London

Ajaiyeoba EO (2000) Phytochemical and antimicrobial studies of *Gynandropsis gynandra* and *Buchholzia coriaceae* extracts. Afr J Biomed Res 3:161–165

Ajaiyeoba EO, Onocha PA, Nwozo SO, Sama W (2003) Antimicrobial and cytotoxicity evaluation of *Buchholzia coriacea* stem bark. Fitoterapia 74:706–709

Ajibesin KK, Ekpo BA, Bala DN, Essien EE, Adesanya SA (2008) Ethnobotanical survey of Akwa Ibom State of Nigeria. J Ethnopharmacol 115:387–408

Ajibola AO, Satake M (1992) Contributions to the phytochemistry of medicinal plants growing in Nigeria as reported in the 1979–1990 literature—a preview. Afr J Pharm Sci 22:172–201

Akah PA, Nwambie AI (1994) Evaluation of Nigerian traditional medicines: 1. plants used for rheumatic (inflammatory) disorders. J Ethnopharmacol 42:179–182

Akihisa T, Gebreyesus T, Hayashi H, Tamura T (1992) Co-occurrence of C-24 epimeric 24-ethylsterols possessing and lacking a Δ25-bond in *Kalanchoe petitiana*. Phytochemistry 31(1):163–166

Akoto O, Oppong IV, Addae-Mensah I, Waibel R, Achenbach H (2008) Isolation and characterization of dipeptide derivative and phytosterol from *Capparis tomentosa* Lam. Sci Res Essay 3(8):355–358

Aladesanmi AJ, Iwalewa EO, Adebajo AC, Akinkunmi EO, Taiwo BJ, Olorunmola FO, Lamikanra A (2007) Antimicrobial and antioxidant activities of some Nigerian medicinal plants. Afr J Tradit Complement Altern Med 4:173–184

Alemayehu G, Hailu A, Abegaz MB (1996) Bianthraquinones from *Senna didymobotrya*. Phytochemistry 42(5):1423–1425

Almora K, Pino JA, Hernández M, Duarte C, González J, Roncal E (2004) Evaluation of volatiles from ripening papaya (*Carica papaya* L., var. Maradol roja). Food Chem 86(1):127–130

Al-Rehaily JA, Ahmad SM, Muhammad I, Al-Thukair AA, Perzanowski PH (2003) Furoquinoline alkaloids from *Teclea nobilis*. Phytochemistry 64:1405–1411

Alvarez GG, Burns BF, Desjardins M, Salahudeen SR, AlRashidi F, Cameron DW (2006) Blastomycosis in a young African man presenting with a pleural effusion. Can Respir J 13(8):441–444

Alves PM, Queiroz LM, Pereira JV, Pereira Mdo S (2009) *In vitro* antimicrobial, antiadherent and antifungal activity of Brazilian medicinal plants on oral biofilm microorganisms and strains of the genus *Candida*. Rev Soc Bras Med Trop 42:222–224

Amos S, Gamaniel K, Akah P, Wambebe C (1998) Anti-inflammatory and muscle relaxant effects of the aqueous extract of *Pavetta crassipes* leaves. Fitotherapia 69:425–429

Ampel NM (1996) Emerging disease issues and fungal pathogens associated with HIV infection. Emerg Infect Dis 2:109–116

Amusan OGO, Adesogan KE, Makinde MJ (1996) Antimalarial active principles of *Spathodea campanulata* stem bark. Phytotherapy Res 10:692–693

Apers S, De Bruyne ET, Claeys M, Vlietinck JA, Pieters ACL (1999) New acylated triterpenoid saponins from *Maesa lanceolata*. Phytochemistry 52:1121–1131

Appidi JR, Grierson DS, Afolayan AJ (2008) Ethnobotanical study of plants used for the treatment of diarrhoea in the Eastern Cape, South Africa. Pakistan J Biol Res 11:1961–1963

Aref HL, Salah KB, Chaumont J, Fekih A, Aouni M, Said K (2010) *In vitro* antimicrobial activity of four *Ficus carica* latex fractions against resistant human pathogens (antimicrobial activity of *Ficus carica* latex). Pak J Pharm Sci 23:53–58

Arif T, Bhosale JD, Kumar N, Mandal TK, Bendre RS, Lavekar GS, Dabur R (2009) Natural products—antifungal agents derived from plants. J Asian Nat Prod Res 11:621–638

Arima H, Danno GI (2002) Isolation of antimicrobial compounds from guava (*Psidium guajava* L.) and their structural elucidation. Biosci Biotechnol Biochem 66(8):1727–1730

Arnold HJ, Gulumian M (1984) Pharmacopoeia of traditional medicine in Venda. J Ethnopharmacol 12:35–74

Arthur J, Hosseinipour MC (2010) Management of cryptococcal meningitis in Sub-Saharan Africa. Curr HIV/AIDS Rep 7(3):134–142

Asres K (1986) Alkaloids and flavonoids from the species of leguminoseae, Thesis presented for the Degree of Philosophy in the University of London, Department of Pharmacognosy, School of Pharmacy, London, pp 49–52

Asres K, Bucar F, Kartnig T, Witvrouw M, Pannecoupue C, de Clerc E (2001a) Antiviral activity against human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) of ethnobotanically selected Ethiopian medicinal plants. Phytother Res 15:62–69

Asres K, Bucar F, Knauder E, Yardley V, Kendrick H, Croft SL (2001b) *In vitro* antiprotozoal activity of extract and compounds from the stem bark of *Combretum molle*. Phytother Res 15(7):613–617

Atawodi S, Mende P, Pfundstein B, Preussmann R, Spiegelhalder B (1995) Nitrosatable amines and nitrosamide formation in natural stimulants *Cola acuminate*, *C. nitida*, and *Garcinia cola*. Food Chem Toxicol 33:625–630

Auvin C, Lezenven F, Blond A, Augeven-Bour I, Pousset JL, Bodo B (1996) Mucronine J a 14-membered cyclopeptide alkaloid from *Zizyphus mucronata*. J Nat Prod 59(7):676–678

Baba-Moussa F, Akpagana K, Bouchet P (1999) Antifungal activities of seven West African Combretaceae used in traditional medicine. J Ethnopharmacol 66:335–338

Back-Brito GN, Mota AJ, Vasconcellos TC, Querido SM, Jorge AO, Reis AS, Balducci I, Koga-Ito CY (2009) Frequency of *Candida* spp. in the oral cavity of Brazilian HIV-positive patients and correlation with CD4 cell counts and viral load. Mycopathologia 67(2):81–87

Baily GG (1989) Blastomycosis. In: Hay RJ (ed) Tropical fungal infections. Bailliere Tindall, London, pp 153–167

Baldé MA, Apers A, De Bruyne ET, Van Den Heuvel H, Claeys M, Vlietinck JA, Pieters CAL (2000) Steroids from *Harrisonia abyssinica*. Planta Med 66(1):67–69

Banno N, Akihisa T, Yasukawa K, Tokuda H, Tabata K, Nakamura Y, Nishimura R, Kimura Y, Suzukc T (2006) Anti-inflammatory activities of the triterpene acids from the resin of *Boswellia carteri*. J Ethnopharmacol 107:249–253

Barra A, Coroneo V, Dessi S, Cabras P, Angioni A (2007) Characterization of the volatile constituents in the essential oil of *Pistacia lentiscus* L. from different origins and its antifungal and antioxidant activity. J Agr Food Chem 55:7093–7098

Barros MDDL, Paes RDA, Schubach AO (2011) *Sporothrix schenckii* and Sporotrichosis. Clin Microbiol Rev 24(4):633–654

Bashwira S, Hootele C (1988) Myricoidine and dihydromyricoidine, two new macrocyclic spermidine alkaloids from *Clerodendrum myricoides*. Tetrahedron 44(14):4521–4526

Batawila K, Kokou K, Koumaglo K, Gbeassor M, de Foucault B, Bouche PH, Akpagana K (2005) Antifungal activities of five combretaceae used in Togolese traditional medicine. Fitoterapia 76:264–268

Beentje HJ (1994) Kenya trees, shrubs and lianas. National Museums of Kenya, Nairobi

Bellakhdar J (1997) La pharmacopée marocaine traditionnelle; Médecine arabe et ancienne et savoirs populaires, Ibis Press, Saint Etienne

Bello AI, Ndukwe IG, Audu TO, Habila DJ (2011) A bioactive flavonoid from *Pavetta crassipes* K. Schum. Org Med Chem Lett 1(1):1–5

Bergaoui A, Boughalleb N, Ben Jannet H, Harzallah-Shiric F, El Mahjoub M, Mighri Z (2007) Chemical composition and antifungal activity of volatiles from three opuntia species growing in Tunisia. Pak J Biol Sci 10(15):2485–2489

Blignaut E (2007) Oral candidiasis and oral yeast carriage among institutionalised South African paediatric HIV/AIDS patients. Mycopathologia 163(2):67–73

Bokesch HR, Charan RD, Merangelman KM, Beutler JA, Gardella R, O'keefe BR, Mekee TC, Memahon JB (2004) Isolation and characterization of anti-HIV peptides from *Dorstenia contrajerva* and *Treculia obovoidea*. FEBS Lett 2004(567):287–290

Borrelli F, Milic N, Ascione V, Capasso R, Izzo AA, Capasso F, Petrucci F, Valente R, Fattorusso E, Taglialatela-Scafati O (2005) Isolation of new rotenoids from *Boerhaavia diffusa* and evaluation of their effect on intestinal motility. Planta Med 71(10):928–932

Botta B, Monache DF, Monache DG, Bettolo MGB, Msonthi JD (1985) Prenylated bianthrones and vismione F from *Psorospermum febrifugum*. Phytochemistry 24(4):827–830

Boukef MK (1986) *Médecine traditionnelle et pharmacopée. Les plantes dans la médecine traditionnelle tunisienne*, Agence de coopération culturelle et technique, Paris, France

Bouquet A (1969) Féticheurs et médecines traditionnelles du Congo Brazzaville. Orstom, Paris, pp 178–202

Bouquet A and Debray M (1974) Plantes medicinales de la Cote d'Ivoire. ORSTOM, Paris

Braide VB (1991) Pharmacological effect of chronic ingestion of *Garcinia kola* seeds in rats. Phytother Res 4:39–41

Brine ND, Campbell WE, Bastida J, Herrera MR, Viladomat F, Codina C, Smith PJ (2002) A dinitrogenous alkaloid from *Cyrtanthus obliquus*. Phytochemistry 61:443–447

Brookes KB, Katsoulis LC (2006) Bioactive components of *Rhoicissus tridentata*: a pregnancy-related traditional medicine. S Afr J Sci 102(5–6):267–272

Bruneton J (2009) Pharmacognosie. Phytochimie. Plantes médicinales, 4^{e} édition, Lavoisier, Paris

Bruneton J (1999) Pharmacognosie: Phytochimie, Plantes medicinales, 3rd edn. Tec & Doc, Paris, pp 263–309

Burkill HM (1985) The useful plants of west tropical Africa. Families M–R, Vol 4. Royal Botanic Garden, Kew

Buswell JA, Chang ST (1993) Edible mushrooms. Attributes and applications. In: Chang ST, Buswell JA, Miles PG (eds) Genetics and breeding of edible mushrooms. Gordon and Breach, Philadelphia, pp 297–394

Buwa LV, Van Staden J (2006) Antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South Africa. J Ethnopharmacol 103:139–142

Byamukama R, Kiremire TB, Andersen MO, Steigen A (2005) Anthocyanins from fruits of *Rubus pinnatus* and *Rubus rigidus*. J Food Compos Anal 18:599–605

Canini A, Alesiani D, D'Arcangelo G, Tagliatesta P (2007) Gas chromatography–mass spectrometry analysis of phenolic compounds from *Carica papaya* L. leaf. J Food Compos Anal 20:584–590

Carman WF, Frean JA, Crewe-Brown HH, Culligan GA, Young CN (1989) Blastomycosis in Africa a review of known cases diagnosed between 1951 and 1987. Mycopathologia 107:25–32

Cheikh RS, Racil H, Ismail O, Trabelsi S, Zarrouk M, Chaouch N, Hantous S, Khaled S, El Mezni F, Chabbou A (2008) Pulmonary blastomycosis: a case from Africa. Sci World J 8:1098–1103

Chen L (1991) Polyphenols from leaves of *Euphorbia hirta* L. Zhongguo Zhong Yao Za Zhi 16(1):38–39

Chhabra SC, Mahunnah RLA, Mshiu EN (1991) Plants used in traditional medicine in eastern Tanzania. V. Angiosperms (Passifloraceae to Sapindaceae). J Ethnopharmacol 33:143–157

Chian-Yong L, Coleman R (2011) Emerging fungal infections in immunocompromised patients. F1000 Med Rep 3:14. doi:10.3410/M3-14

Chin YW, Mdee KL, Mbwambo HZ, Mi Q, Chai HB, Cragg MG, Swanson MS, Kinghorn DA (2006) Prenylated flavonoids from the root bark of *Berchemia discolor*, a Tanzanian medicinal plant. J Nat Prod 69(11):1649–1652

Comoe L (1987) Apports de la medecine traditionnelle Africaine à la chimiotherapie anticancereuse: Activite antitumorale et differenciante de la Fagaronine (*Fagara xanthoxyloides* Lam.), These de Doctorat d'Etat es-Sciences pharmaceutiques, Universite de Reims, pp 26–52

Conrad J, Vogler B, Klaiber I, Reeb S, Guse JH, Roos G, Kraus W (2001) Vanillic acid 4-O-β-D-(6′-O-galloyl) Glucopyranoside and other constituents from the bark of *Terminalia macroptera* guill. et perr. Nat Prod Lett 15(1):35–42

Cos P, Hermans N, DeBruyne T, Apers S, S indambiwe JB, Vanden Berghe D, Pieters L, Vlietinck AJ (2002) Further evaluation of Rwandan medicinal plant extracts for their antimicrobial and antiviral activities. J Ethnopharmacol 79:155–163

Dalziel JM (1937) The useful plants of West Tropical Africa, The crown Agents for the Colonies, London, pp 346–349

De las Heras B, Rodríguez B, Boscá L, Villar AM (2003) Terpenoids: sources, structure elucidation and therapeutic potential in inflammation. Curr Topics Med Chem 3:171–185

De Tommasi N, Piacente S, de Simone F, Pizza C (1993) New sucrose derivatives from the bark of *Securidaca longipedunculata*. J Nat Prod 56:134–137

Deborah KBR, Mecky INM, Olipa DN, Cosam CJ, Zakaria HM (2006) Screening of Tanzanian medicinal plants for anti-candida activity. BMC Complement Altern Med 6:11

Dekebo A, Dagne E, Gautun RO, Aasen JA (2002) Triterpenes from the resin of *Boswellia neglecta*. Bull Chem Soc Ethiop 16(1):87–90

Delaveau P, Desvignes A, Adoux E, Tessier AM, Chen D (1979) Baguettes frotte-dents d'Afrique occidentale, tri chimique et microbiologique. Annales Pharmaceutiques Française 37:185–190

Delazar A, Gibbons S, Kosari AR, Nazemiyeh H, Modarresi M, Nahar L, Sarker DS (2006) Flavone c-glycosides and cucurbitacin glycosides from *Citrullus colocynthis*. DARU J Pharm Sci 14(3):109–114

Desta B (1993) Ethiopian traditional herbal drugs. Part II: antimicrobial activity of 63 medicinal plants. J Ethnopharmacol 39:129–139

Diallo D, Diarra N, Keita A, Doumbia O (1991) Contribution à l'etude du Traitement Traditionnel des Brulures, des Plaies et des Otites dans Trois Localites du Mali: Bamako, Narena, Siby, INRSP/DMT, Bamako

Diallo D (2000) Ethnopharmacological survey of medicinal plants in Mali and phytochemical study of four of them: *Glinus oppositifolius* (aizoaceae), *Diospyros abyssinica* (ebenaceae), *Entada africana* (mimosaceae), *Trichilia emetica* (meliaceae). These de Doctorat. Faculté des Sciences, Université de Lausanne, Switzerland

Diallo D, Hveem B, Mahmoud MA, Berge G, Paulsen BS, Maiga A (1999) An ethnobotanical survey of herbal drugs of Gourma district mali. Pharm Biol 37:80–91

Diallo D, Marston A, Terreaux C, Toure Y, Paulsen BS, Hostettmann K (2001) Screening of malian medicinal plants for antifungal, larvicidal, molluscicidal, antioxidant and radical scavenging activities. Phytother Res 15:401–406

Diamond RD (1991) The growing problem of mycoses in patients infected with the human immunodefficiency virus. Rev Infect Dis 13(3):480–486

Djemgou PC, Gatsing D, Kenmogne M, Ngamga D, Aliyu R, Adebayo HA, Tane P, Ngadjui TB, Seguin E, Adoga IG (2007) An antisalmnellal agent and a new dihydroanthracenone from *Cassia petersiana*. Res J Med Plant 1(2):65–71

Djoukeng JD, Abou-Mansour E, Tabacchi R, Tapondjou AL, Bouda H, Lontsi D (2005) Antibacterial triterpenes from *Syzygium guineense* (myrtaceae). J Ethnopharmacol 101:283–286

Dramane KL and Houngnon P (1986) Usages therapeutiques de quelques espèces de Combretaceae du genre Combretum dans la Pharmacopée traditionnelle beninoise. Plantes Medicinales et Phytotherapie 20:264–271

Duker-Eshun G, Jaroszewski WJ, Asomaning AW, Oppong-Boachie F, Christensen BS (2004) Antiplasmodial constituents of *Cajanus cajan*. Phytother Res 18:128–130

Dzoyem JP, Kechia FA, Pieme AC, Kuete V, Akak CM, Tangmouo JG, Lohoue PJ (2011) Phytotoxic, antifungal activities and acute toxicity studies of crude extract and compounds from *Diospyros canaliculata*. Nat Prod Res 25:741–749

Dzoyem JP, Tangmouo JG, Kechia FA, Lontsi D, Etoa FX, Lohoue PJ (2006a) In vitro antidermatophytic activity of *Diospyros crassiflora* hiern (ebenaceae). Sudan J Dermatol 4:10–15

Dzoyem JP, Tangmouo JG, Lontsi D, Etoa FX, Lohoue PJ (2007) In vitro antifungal activity of extract and plumbagin from *Diospyros crassiflora* hiern (ebenaceae). Phytother Res 21:671–674

Dzoyem JP, Tangmouo JG, Manfouo R, Lontsi D, Etoa FX, Lohoue PJ (2006b) Antifungal activity of some Cameroonian medicinal plants. Nige J Nat Prod Med 10:94–96

Eboi E, Doukoure B, Kolia DP, Aoussi E, Koffi E, Doumbia A, Kouadio K, Eholie S, Bissagnene E (2011) Intestinal histoplasmosis with *Histoplasma duboisii* in a patient infected by HIV-1 in Abidjan (Ivory Coast). J AIDS Clin Res 2(5):125

El Euch D, Cherif F, Aoun K, Mokni M, Bouratbine A, Haouet S, Ben Osman Dhahri A (2004) Cutaneous blastomycosis: description of two cases in Tunisia. Médecine Tropicale: revue du Corps de Santé colonial 64(2):183–186

Eldeen IMS, Elgorashi EE, Mulholland DA, Van Staden J (2006) Anolignan B: a bioactive compound from the roots of *Terminalia sericea*. J Ethnopharmacol 103:135–138

Elhadj SB, Megalizzi V, Traoréa MS, Cos P, Maes L, Decaestecker C, Pieters L, Baldé AM (2010) In vitro antiprotozoal, antimicrobial and antitumor activity of *Pavetta crassipes* K. Schum leaf extracts. J Ethnopharmacol 130:529–535

Elin M, Diallo D, Oyvind MA, Karl EM (2002) Antioxidants from the bark of *Burkea africana*, an African medicinal plant. Phytotherapy Res 16:148–153

Enwuru CA, Ogunledun A, Idika N, Enwuru NV, Ogbonna F, Aniedobe M, Adeiga A (2008) Fluconazole resistant opportunistic oro-pharyngeal *Candida* and non-*Candida* yeast-like isolates from HIV infected patients attending ARV clinics in Lagos. Niger Afr Health Sci 8(3):142–148

Erasto P, Grierson DS, Afolayan AJ (2006) Bioactive sesquiterpene lactones from the leaves of *Vernonia amygdalina*. J Ethnopharmacol 106(1):117–120

Eyong KO, Folefoc GN, Kuete V, Beng VP, Krohn K, Hussain H, Nkengfack AE, Saeftel M, Sarite SR, Hoerauf A (2006) Newbouldiaquinone A: a naphthoquinone–anthraquinone ether coupled pigment, as a potential antimicrobial and antimalarial agent from *Newbouldia laevis*. Phytochemistry 67:605–609

Fale LP, Borges C, Madeira APJ, Ascensão L, Araujo MME, Florêncio MH, Serralheiro MML (2009) Rosmarinic acid, scutellarein 40-methyl ether 7- O -glucuronide and (16S)-coleon E are the main compounds responsible for the antiacetylcholinesterase and antioxidant activity in herbal tea of *Plectranthus barbatus* (falso boldo). Food Chem 114:798–805

Fan-Harvard P, Capano D, Smith SM, Mangia A, Eng HRH (1991) Development of resistance in *Candida* isolates from patients receiving prolonged antifungal therapy. Antimicrob Agents Chemother 35:2302–2305

Faskhutdinov MF, Telezhenetskaya MV, Levkovich MG, Abdullaev ND (2000) Alkaloids of *Peganum harmala*. Chem Nat Compd 36(6):602–605

Fatima H, Claude A, Annick A, Mahammed A (2002) Effects of wounding and salicylic acid on hydroxycinnamoylmalic acids in *Thunbergia alata*. Plant Physiol Biochem 40(9):761–769

Ferchichia L, Mekni A, Bellil K, Haouet S, Zeddini A, Bellil S, Kchir N, Cherif F, Ben Osman A, Zitouna M (2006) Three cases of cutaneous blastomycosis. Medecine et Maladies Infectieurses 36(5):285–287

Fiot J, Sanon S, Azas N, Mahiou V, Jansen O, Angenot L, Balansard G, Ollivier E (2006) Phytochemical and pharmacological study of roots and leaves of *Guiera senegalensis* J.F. Gmel (Combretaceae). J Ethnopharmacol 106:173–178

Fullas F (2001) Ethiopian traditional medicine: common medicinal plants in perspective, 1st edn. Library Congress Cataloging, Iowa

Fyhrquist P, Mwasumbi L, Hæggstro CA, Vuorela H, Hiltunen R, Vuorela P (2002) Ethnobotanical and antimicrobial investigation on some species of *Terminalia and Combretum* (Combretaceae) growing in Tanzania. J Ethnopharmacol 79:169–177

Gaitán I, Paz AM, Zacchino SA, Tamayo G, Giménez A, Pinzón R, Cáceres A, Gupta MP (2011) Subcutaneous antifungal screening of Latin American plant extracts against *Sporothrix schenckii* and *Fonsecaea pedrosoi*. Pharm Biol 49:907–919

Garcia-Guinon A, Torres-Rodriguez JM, Ndidongarte DT, Cortadellas F, Labrin L (2009) Disseminated histoplasmosis by *Histoplasma capsulatum* var. *duboisii* in a paediatric patient from the Chad Republic, Africa. Eur J Clin Microbiol Infect Dis 28(6):697–699

Geuns JMC (1978) Steroid hormones and plant growth and development. Phytochemistry 17:1–14

Gomah N (2010) Antibacterial and antifungal activities of β-carboline alkaloids of *Peganum harmala* (L) seeds and their combination effects. Fitoterapia 81:779–782

Gugnani HC (2000) Histoplasmosis in Africa: a review. Ind J Chest Dis Allied Sci 42:271–277

Gumbo T, Just-Nübling G, Robertson V, Latif AS, Borok MZ, Hohle R (2001) Clinicopathological features of cutaneous histoplasmosis in human immunodeficiency virus-infected patients in Zimbabwe. Trans Roy Soc Trop Med Hyg 21:54–56

Gumbo T, Kadzirange G, Mielke J, Gangaido IT, Hakim JG (2002) *Cryptococcus neoformans* meningoencephalitis in African patients with acquired immunodeficiency syndrome. Pediatr Infect Dis J 21:54–56

Gundidza M, Sibanda M (1991) Antimicrobial activities of *Ziziphus abyssinica* and *Berchemia discolour*. Central Afr J Med 37:80–83

Gustafsson MHG, Bittrich V, Stevens PF (2002) Phylogeny of clusiaceae based on rbcL sequences. Int J Plant Sci 163(6):1045

Hailu T, Endris M, Kaleab A, Tsige GM (2005) Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders. J Ethnopharmacol 100:168–175

Hakizamungut E, Van Puyveldet L, Wéry M, De Kimpe N, Schamp N (1988) active principles of *Tetradenia riparia* III. Anti-trichomonas activity of 8(14),15-sandaracopimaradiene-7α, 18-diol. Phytother Res 2(4):207–208

Hamill FA, Apio S, Mubiru NK, Bukenya-Ziraba R, Mosango M, Maganyi OW, Soejarto DD (2003) Traditional herbal drugs of Southern Uganda, II: literature analysis and antimicrobial assay. J Ethnopharmacol 84:57–78

Hamza OJ, van den Bout-van den Beukel CJ, Matee MI, Moshi MJ, Mikx FH, Selemani HO, Mbwambo ZH, Van der Ven AJ, Verweij PE (2006) Antifungal activity of some Tanzanian plants used traditionally for the treatment of fungal infections. J Ethnopharmacol 108:124–132

Harinantenaina L, Kasai R, Yamasaki K (2002) Cussosaponins A-E, triterpene saponins from the leaves of *Cussonia racemosa*, a malagasy endemic plant. Chem Pharm Bull 50:1290–1293

Harket A, Oukabli M, Al Bouzidi A, Zoubeir Y, Quamous O, Baba N, Doghmi K, Mikdame M, Rimani M, Sedrati O, Labraimi A (2007) Cutaneous blastomycosis revealing intravascular B-cell lymphoma: a case in Morocco. Médecine Tropicale: revue du Corps de Santé colonial 67(3):278–280

Harrison TS (2009) The burden of HIV-associated cryptococcal disease. AIDS 23:531–532

He W, Van Puyvelde L, Bosselaers J, De Kimpe N, Van Der Flaas M, Roymans A, Mathenge SG, Mudida FP, Chalo MPB (2002) Activity of 6-pentadecylsalicylic acid from *Ozoroa insignis* against marine crustaceans. Pharmaceut Biol 40:74–76

Hedberg I, Hedberg O (1982) Inventory of plants used in traditional medicine in Tanzania. I. Plants of the families acanthaceae-cucurbitaceae. J Ethnopharmacol 6:29–60

Hedberg I, Hedberg O, Madati PJ, Mshigeni KE, Mshiu EN, Samuelsson G (1983) Inventory of plants used in traditional medicine in Tanzania. part III. plants of the families papilionaceae-vitaceae. J Ethnopharmacol 9:237–260

Hiroshi K, Yoshitsugu M, Hisato S, Tsutomu K, Masafumi S, Koichi I, Yoshihiro Y, Kazunori Y, Takayoshi T, Shigeru K (2008) Efficacy of SPK-843, a novel polyene antifungal, in a murine model of systemic cryptococcosis. Antimicrob Agents Chemother 52(5):1871–1872

Houghton PJ, Hylands PJ, Mensah AY (2005) In vitro tests and ethnopharmacological investigations: wound healing as an example. J Ethnopharmacol 100:100–107

http://en.wikipedia.org/wiki/Euphorbiaceae. Euphorbiaceae. Accessed on Jan 02 2012

Hutchings A, Scott AH, Lewis G, Cunningham A (1996) Zulu medicinal plants: an inventory. University of Natal Press, Scotts ville

Ikhiri K, Mahaman I, Ahond A, Chiaroni A, Poupat C, Riche C, Potier P (1995) Le limeolide, nouveau sesquiterpene de type drimane isolé de *Limeum pterocarpum*. J Nat Prod 58(7):1136–1138

Irvine JR (1961) Woody plants of Ghana, 2nd edn. Oxford University press, London

Iwalokun BA, Efedede BU, Alabi-Sofunde JA, Oduala T, Magbagbeola OA, Akinwande AI (2006) Hepato protective and antioxidant activities of *Vernonia amygdalina* on acetaminophen induced hepatic damage in mice. J Med Food 9:524–539

Iwu MM, Igboko OA, Okunji CO, Tempesta MS (1990) Antidiabetic and aldose reductase activities of biflavanones of *Garcinia kola*. J Pharm Pharmacol 42:290–292

Jagtap UB, Bapat VA (2010) Artocarpus: a review of its traditional uses, phytochemistry and pharmacology. J Ethnopharmacol 129:142–166

Jeruto P, Lukhoba C, Ouma G, Otieno D, Mutai C (2008) An ethnobotanical study of medicinal plants used by the Nandi people Kenya. J Ethnopharmacol 116:370–376

Johann S, Cisalpino PS, Watanabe GA, Cota BB, de Siqueira EP, Pizzolatti MG, Zani CL, de Resende MA (2010) Antifungal activity of extracts of some plants used in Brazilian traditional medicine against the pathogenic fungus *Paracoccidioides brasiliensis*. Pharm Biol 48:388–396

Jonathan SG, Fasidi IO (2003) Antimicrobial activities of two Nigerian edible macro-fungi *Lycoperdon pusilum* (bat. Ex) and *Lycoperdon giganteum* (Pers.). Afr J Biomed Res 6:85–90

Joseph CC, Moshi MJ, Sempombe J, Nkunya MHH (2006) (4-methoxy-benzo[1,3]dioxol-5-yl)-phenylmethanone: an antibacterial benzophenone from *Securidaca longepedunculata*. Afr J Tradit Complement Altern Med 3:80–86

Jossang A, Pousset JL, Bodo B (1994) Combreglutinin, a hydrolyzable tannin from *Combretum glutinosum*. J Nat Prod 57:732–737

Jossang A, Seuleiman M, Maidou E, Bodo B (1996) Pentacyclic triterpenes from *Combretum nigricans*. Phytochemistry 41(2):591–594

Julião DSL, Leitão GS, Lotti C, Picinelli LA, Rastrelli L, Fernandes DP, Noël F, Thibaut BJP, Leitão GG (2010) Flavones and phenylpropanoids from a sedative extract of *Lantana trifolia* L. Phytochemistry 71:294–300

Kabangu L, Galeffi C, Aonzo E, Nicoletti M, Messana I (1987) New biflavone from the bark of *Garcinia Kola*. Planta Med 11:275–277

Kambu K (1990) Elément de phytothérapie comparée: plantes médicinales africaines, C.R.P., Kinshasa, Zaire

KamwendoWY, Chiotha SS, Msonthi JD (1985) Screening of plants used traditionally to control shistosomiasis in Malawi. Fitoterapia 56:229–232

Kareem SO, Akpan I, Ojo OP (2008) Antimicrobial activities of *Calotropis procera* on selected pathogenic microorganisms. Afr J Biomed Res 11:105–110

Kareru PG, Gachanja AN, Keriko JM, Kenji GM (2008) Antimicrobial activity of some medicinal plants used by herbalists in eastern province Kenya. Afr J Tradit Complement Altern Med 5:51–55

Kathleen JM, Govender NP, Rossouw J, Meiring S, Mitchell TG (2011) Analyses of pediatric isolates of *Cryptococcus neoformans* from South Africa. J Clin Microbiol 49(1):307–314

Katsayal UA, Abdurahman EM (2002) Pharmacognostic studies on the leaves of *Pavetta crassipes*. Nig J Nat Prod Med 6:30–32

Kerharo J and Adam JG (1974) *La pharmacope Senegalaise traditionnelle*: *Plantes medicinales et toxiques*. Edition Vigot-freres, Paris

Kerharo J and Bouquet J (1950) Plantes Medicinales de la Cote d′Ivoire et Haute Volta, Vigot Eds, Paris

Kernan MR, Amarquaye A, Chen JL, Chan J, Sesin DF, Parkinson N, Ye Z-J, Barrett M, Bales C, Stoddart CA, Sloan B, Blanc P, Limbach C, Mrisho S, Rozhon EJ (1998) Antiviral phenylpropanoid glycosides from the medicinal plant *Markhamia lutea*. J Nat Prod 61:564–570

Kew F (1985) The useful plants of West Tropical Africa, Families A-D 2nd edn, Burkill, H. M. Royal Botanical Gardens, pp 219–222

Khan QA, Malik A (1989) A steroid from *Calotropis procera*. Phytochemistry 28:2859–2861
Kisangu DP, Hosea KM, Joseph CC, Lyaruu HVM (2007) In vitro antimicrobial assay of plants used in traditional medicine in Bukoba rural district, Tanzania. Afr J Tradit Complement Altern Med 4:510–523
Kloos H, Tekle A, Yohannes LW, Yosef A, Lemma A (1978) Preliminary studies on traditional medicinal plants in nineteen markets in Ethiopia: use patterns and public health aspects. Ethiop Med J 16:33–43
Kokwaro JO (1976) Medicinal plants of East Africa. East African Literature Bureau, Nairobi
Kone M, Atindehou KK, Traoré D (2002) Plantes et médecine traditionnelle dans la région de Ferkessédougou (Côte d'Ivoire). Annales de Botanique de l'Afrique de l'Ouest 2:13–21
Koudou J, Ossibi EAW, Aklikokou K, Abena AA, Gbeassor M, Bessière JM (2007) Chemical composition and hypotensive effects of essential oil of *Monodora myristica* Gaertn. J Biol Sci 7(6):937–942
Kubo I, Kim M, Ganjiau I (1987) Isolation, structure and synthesis of maesanin, a host defense stimulant from an african medicinal plant *Maesa lanceolata*. Tetrahedron 43:2653–2660
Kuete V (2005) Evaluation des propriétés antimicrobiennes de deux plantes médicinales Camerounaises utilises dans le traitement des maladies infectieuses : *Solanum trvum* (solanaceae) et *Tabernaemontana crassa* (apocynaceae). Thèse de Doctorat/PhD. Université de Yaoundé I; 2005
Kuete V, Efferth T (2010) Cameroonian medicinal plants: pharmacology and derived natural products. Frontiers Pharmacol 1:1–19
Kuete V (2010) Potential of cameroonian plants and derived-products against microbial infections: a review. Planta Med 76:1479–1491
Kuete V, Eyong KO, Beng VP, Folefoc GN, Hussain H, Krohn K, Nkengfack AE, Saeftel M, Sarite SR, Hoerauf A (2007a) Antimicrobial activity of the methanolic extract and compounds isolated from the stem bark of *Newbouldia laevis* Seem. (Bignoniaceae). Pharmazie 62:552–556
Kuete V, Fozing DC, Kapche WFGD, Mbaveng AT, Kuiate JR, Ngadjui BT, Abegaz BM (2009) Antimicrobial activity of the methanolic extract and compounds from *Morus mesozygia* stem bark. J Ethnopharmacol 124:551–555
Kuete V, Kamga J, Sandjo LP, Ngameni B, Poumale HM, Ambassa P, Ngadjui BT (2011) Antimicrobial activities of the methanol extract, fractions and compounds from *Ficus polita* Vahl. (Moraceae). BMC Complemet Altern Med 11:6
Kuete V, Komguem J, Beng VP, Tangmouo JG, Meli AL, Etoa FX, Lontsi D (2007b) Antimicrobial components of the methanolic extract from the stem bark of *Garcinia smeathmannii* oliver (clusiaceae). S Afr J Bot 73:347–354
Kuete V, Metuno R, Ngameni B, Tsafack AM, Ngandeu F, Fotso GW, Bezabih M, Etoa FX, Ngadjui BT, Abegaz BM, Beng VP (2007c) Antimicrobial activity of the methanolic extracts and compounds from *Treculia obovoidea* (moraceae). J Ethnopharmacol 112:531–536
Kuete V, Metuno R, Ngameni B, Tsafack AM, Ngandeu F, Fotso GW, Bezabih M, Etoa FX, Ngadjui BT, Abegaz BM, Beng VP (2008a) Antimicrobial activity of the methanolic extracts and compounds from *Treculia obovoidea* (moraceae). S Afr J Bot 74:111–115
Kuete V, Ngameni B, Fotso Simo CC, Kengap Tankeu R, Tchaleu Ngadjui B, Meyer JJM, Lall N, Kuiate JR (2008b) Antimicrobial activity of the crude extracts and compounds from *Ficus chlamydocarpa* and *Ficus cordata* (moraceae). J Ethnopharmacol 120:17–24
Kuete V, Ngameni B, Tsafack AM, Ambassa IK, Simo P, Roy R, Bezabih M, Etoa FX, Ngadjui BT, Abegaz BM, Beng VP (2007d) Antimicrobial activity of the extract from the twigs of *Dorstenia elliptica* (moraceae). Pharmacologyonline 1:573–580
Kuete V, Nguemeving JR, Beng VP, Azebaze AGB, Etoa FX, Meyer M, Bodo B, Nkengfack AE (2007e) Antimicrobial activity of the methanolic extracts and compounds from *Vismia laurentii* de wild (guttiferae). J Ethnopharmacol 109:372–379
Kuete V, Simo IK, Beng VP, Bigoga JD, Kapguep RN, Etoa FX, Ngadjui BT (2007f) Antimicrobial activity of the methanolic extract, fractions and four flavonoids from the twigs of *Dorstenia angusticornis* engl. (moraceae). J Ethnopharmacol 112:271–277

Kuete V, Wabo GF, Ngameni B, Tsafack AM, Metuno R, Etoa FX, Ngadjui BT, Beng VP (2007g) Antimicrobial activity of the methanolic extract and compounds from the stem bark of *Irvingia gabonensis* (ixonanthaceae). J Ethnopharmacol 114:54–60

Lahmiti S, Baki S (2009) Thoracic blastomycosis from morocco. Sci World J 9:200

Lall N, Weigan O, Hussein AA, Meyer JJM (2006) Antifungal activity of naphthoquinones and triterpenes isolated from the root bark of *Euclea natalensis*. S Afr J Bot 72:579–583

Latha LY, Darah I, Jain K, Sasidharan S (2011) Effects of *Vernonia cinerea* less methanol extract on growth and morphogenesis of *Candida albicans*. Eur Rev Med Pharmacol Sci 15:543–549

Le Flock E (1983) Contribution à une étude ethnobotanique de la flore tunisienne. Tunis: Imprimerie officielle de la république tunisienne

Lenta NB, Ngouela S, Boyom FF, Tantangmo F, Tchouya FGR, Tsamo E, Gut J, Rosenthal JP, Connolly JD (2007) Anti-plasmodial activity of some constituents of the root bark of *Harungana madagascariensis* LAM. (hypericaceae). Chem Pharm Bull 55:464–467

Leo DM, Braca A, Sanogo R, Cardile V, De Tommasi N, Russo A (2006a) Antiproliferative activity of *Pteleopsis suberosa* leaf extract and its flavonoid components in human prostate carcinoma cells. Planta Med 72(7):604–610

Leo DM, De Tommasi N, Sanogo R, D'Angelo V, Germano PM, Bisignano G, Braca A (2006b) Triterpenoid saponins from *Pteleopsis suberosa* stem bark. Phytochemistry 67:2623–2629

Limmatvapirat C, Sirisopanaporn S, Kittakoop P (2004) Antitubercular and antiplasmodial constituents of *Abrus precatorius*. Planta Med 70(3):276–278

Lin CN, Lu CM, Cheng MK, Gan KH, Won SJ (1990) The cytotoxic principles of *Solanum incanum*. J Nat Prod 53(2):513–516

Lin J, Opolu AR, Geheeb-Keller M, Hutchings AD, Terblanche SE, Jager AK, Van Staden J (1999) Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory anti-microbial activities. J Ethnopharmacol 68:267–274

Linde JH, Combrinck S, Regnier TJC, Virijevic S (2010) Chemical composition and antifungal activity of the essential oils of *Lippia rehmannii* from South Africa. S Afr J Bot 76:37–42

Liu M, Katerere RD, Gray IA, Seidel V (2009) Phytochemical and antifungal studies on *Terminalia mollis* and *Terminalia brachystemma*. Fitoterapia 80:369–373

Liu Y, Murakami N, Ji H, Abreu P, Zhang S (2007) Antimalarial flavonol glycosides from *Euphorbia hirta*. Pharm Biol 45(4):278–281

Lohombo-Ekomba ML, Okusa PN, Penge O, Kabongo C, Iqbal Choudhary M, Kasende OE (2004) Antibacterial, antifungal, antiplasmodial, and cytotoxic activities of *Albertisia villosa*. J Ethnopharmacol 93:331–335

Lohoue P, Lando AJ, Kaptue L, Tchinda V, Folefack M (2004) Superficial mycoses and HIV infection in Yaounde. Eur Acad Dermatol Venereol 18:301–304

Lopes RM, Agostini-Costa TS, Gimenes MA, Silveira D (2011) Chemical composition and biological activities of *Arachis* species. J Agri Food Chem 59:4321–4330

Loulergue P, Bastides F, Baudouin V, Chandenier J, Mariani-Kurkdjian P, Dupont B, Viard JP, Dromer F, Lortholary O (2007) Literature review and case histories of *Histoplasma capsulatum* var. *duboisii* infections in HIV-infected patients. Emerg Infect Dis 13(11):1647–1652

Lukhoba CW, Simmonds MSJ, Paton AJ (2006) Plectranthus: a review of ethnobotanical uses. J Ethnopharmacol 103:1–24

Lumnitzera (2010) Black mangroves. http://www.mangrovewatch.org.au/index.php?option=com_content&view=category&layout=blog&id=38&Itemid=300170. Accessed on Dec 20 2011

Mabogo DEN (1990) The ethnobotany of the Vhavenda, M. Sc. Thesis, University of Pretoria, Pretoria, South Africa

Madhumitha G, Saral AM (2011) Preliminary phytochemical analysis, antibacterial, antifungal and anticandidal activities of successive extracts of *Crossandra infundibuliformis*. Asian Pac J Trop Med 4:192–195

Madubunyi II (1995) Antimicrobial activities of the constituents of Garcinia kola seeds. Int J Pharmacogn 33:232–237

Madureira AM, Ramalhete C, Mulhovo S, Duarte A, Ferreira MJU (2012) Antibacterial activity of some African medicinal plants used traditionally against infectious diseases. Pharm Biol 50(4):481–489

Magassouba FB, Diallo A, Kouyate M, Mara F, Mara O, Bangoura O, Camara A, Traore S, Diallo AK, Zaoro M, Lamah K, Diallo S, Camara G, Traore S, Keita A, Camara MK, Barry R, Keita S, Oulare K, Barry MS, Donzo M, Camara K, Tote K, Vanden Berghe D, Totte J, Pieters L, Vlietinck AJ, Balde AM (2007) Ethnobotanical survey and antibacterial activity of some plants used in Guinean traditional medicine. J Ethnopharmacol 114:44–53

Maiga A, Malterud KE, Mathisen R, Thomas-Oates J, Bergstrom E, Reubsaet L, Diallo D, Paulsen BS (2007) Cell protective antioxidant from the roots of *Lannea velutina* A. Rich, a malian medicinal plant. J Med Plants Res 1(4):066–079

Maima AO, Thoithi GN, Ndwigah SN, Kamau FN, Kibwage IO (2008) Phytosterols from the stem bark of *Combretum fragrans* F. Hoffm. East central Afr J Pharm Sci 11(2):34–39

Mainen JM, Zakaria HM, Modest CK, Viva HM, Chacha M (2006) Antimicrobial and brine shrimp lethality of extracts of *Terminalia mollis* laws. Afr J Tradit Complement Altern Med 3:59–69

Marzouk BZ, Zohra M, Rachel D, Hayet E, Ehsen H, Nadia F, Mahjoub A (2009) Antibacterial and anticandidal screening of Tunisian *Citrullus colocynthis* schrad fFrom medenine. J Ethnopharmacol 125:344–349

Marzouk B, Marzouk Z, Décor R, Mhadhebi L, Fenina N, Aouni M (2010) Antibacterial and antifungal activities of several populations of Tunisian *Citrullus colocynthis* schrad immature fruits and seeds. J Mycol Med 20:179–184

Mashimbye JM, Maumela CM, Drewes ES (1999) A drimane sesquiterpenoid lactone from *Warburgia salutaris*. Phytochemistry 51:435–438

Masoko P, Picard J, Eloff JN (2005) Antifungal activities of six South African *Terminalia* species (combretaceae). J Ethnopharmacol 99:301–308

Mathias ME (1982) Some medicinal plants of the Hehe tribe (southern highlands province, Tanzania). Taxon 31:488–494

Mbaveng AT, Kuete V, Nguemeving JR, Krohn K, Nkengfack AE, Meyer JJM, Lall N (2008a) Antimicrobial activity of the extracts and compounds from *Vismia guineensis* (guttiferae). Asian J Tradit Med 3:211–223

Mbaveng AT, Ngameni B, Kuete V, Konga Simo I, Ambassa P, Roy R, Bezabih M, Etoa FX, Ngadjui BT, Abegaz BM, Meyer JJM, Lall N, Beng VP (2008b) Antimicrobial activity of the crude extracts and five flavonoids from the twigs of *Dorstenia barteri* (moraceae). J Ethnopharmacol 116:483–489

Mebazaa A, Oumari KE, Ghariani N, Mili AF, Belajouza C, Nouira R, Denguezli M, Ben Said M (2010) Tinea capitis in adults in Tunisia. Int J Dermatol 49(5):513–516

Mebe PP, Makuhunga P (1992) 11-(E)-p-coumaric acid ester of bergenin from *Peltophorum africanum*. Phytochemistry 31(9):3286–3287

Mensah AY, Houghton PJ, Fleischer TC, Adu C, Agyare C and Ameade AE (2003) Antimicrobial and antioxidant properties of two Ghanaian plants used traditionally for wound healing. J Pharm Pharmacol 55(Supplement):S-4

Mevy JP, Bessiere JM, Rabier J, Dherbomez M, Ruzzier M, Millogo J, Viano J (2006) Composition and antimicrobial activities of the essential oil of *Triumfetta rhomboidea* Jacq. Flavour Fragr J 21:80–83

Mikaeili A, Modaresi M, Karimi I, Ghavimi H, Fathi M, Jalilian N (2012) Antifungal activities of *Astragalus verus* olivier against *Trichophyton verrucosum* on in vitro and in vivo guinea pig model of dermatophytosis. Mycoses 55: 318–325

Mokoka TA, McGaw LJ, Eloff JN (2010) Antifungal efficacy of ten selected South African plant species against *Cryptococcus neoformans*. Pharm Bio 48:397–404

Mosa RA (2011). In vitro anti-platelet aggregation activity of the extracts of *Protorhus longifolia*. Masters of Science thesis, University of Zululand, KwaDlangezwa, South Africa

Moshi MJ, Innocent E, Masimba PJ, Otieno DF, Weisheit A, Mbabazi P, Lynes M, Meachem K, Hamilton A, Urassa I (2009) Antimicrobial and brine shrimp toxicity of some plants used in

traditional medicine in Bukoba District, north-western Tanzania. Tanzanian J Health Res 11:23–28
Moody JO, Robert VA, Connolly JD, Houghton PJ (2006) Anti-inflammatory activities of the methanol extracts and an isolated furanoditerpene constituent of *Sphenocentrum jollyanum* pierre (Menispermaceae). J Ethnopharmacol 104:87–91
Motlhanka DMT, Habtemariam S, Houghton P (2008) Free radical scavenging activity of crude extracts and 4′-O-methylepigallocatechin isolated from roots of *Cassine transvaalensis* Burtt-Davy from Botswana. Afr J Biomed Res 11:55–63
Motsei ML, Lindsey KL, van Staden J, Jäger AK (2003) Screening of traditionally used South African plants for antifungal activity against *Candida albicans*. J Ethnopharmacol 86:235–241
Motswaledi H, Nkosi L, Moloabi C, Ngobeni K, Nemutavhanani D, Maloba B (2011) Sporotrichosis: a case report and literature review. J Clin Exp Dermatol Res 2:132. doi:10.4172/2155-9554.1000132
Moulary B, Pellequer Y, Lboutounne H, Girard C, Chaumont JP, Millet J, Muyard F (2006) Isolation and in vitro antibacterial activity of astilbin, the bioactive flavanone from the leaves of *Harungana madagascariensis* Lam. ex Poir. (hypericaceae). J Ethnopharmacol 106:272–278
Msonthi JD, Magombo D (1983) Medicinal herbs in Malawi and their uses. Hamdard 26:94–100
Mujovo FS, Hussein AA, Meyer MJJ, Fourie B, Muthivhi T, Lall N (2008) Bioactive compounds from *Lippia javanica* and *Hoslundia opposite*. Nat Prod Res 22(12):1047–1054
Muregi WF, Ishih A, Miyase T, Suzuki T, Kino H, Amano T, Mkoji MG, Mamoru T (2007) Antimalaria activity of methanolic extracts from plants used in Kenyan ethnomedicine and their interactions with chloroquine (CQ) against a CQ-tolerant rodent parasite, in mice. J Ethnopharmacol 111:190–195
Nadembega P, Boussim JI, Nikiema JB, Ferruccio P, Fabiana A (2011) Medicinal plants in Baskoure, Kourittenga province, Burkina Faso: an ethnobotanical study. J Ethnopharmacol 133:378–395
Nartey F, Brimer L, Christensen SB (1981) Proacaciberin, a cyanogenic glycoside from *Acacia sieberiana* var. *Woodii*. Phytochemistry 20(6):1311–1314
Nath D, Sethi N, Srivastava R, Jain AK, Singh RK (1993) Study on tetragenic and antifertility activity of *Peganum harmala* in rats. Fitoterapia 64:321–324
Nawwar M, Hussein S, Ayoub N, Hashim A, El-Sharawy R, Lindequist U, Harms M, Wende K (2011) Constitutive phenolics of *Harpephyllum caffrum* (anacardiaceae) and their biological effects on human keratinocytes. Fitoterapia 82:1265–1271
Nazaruk J, Karna E, Wieczorek P, Sacha P, Tryniszewska E (2010) In vitro antiproliferative and antifungal activity of essential oils from *Erigeron acris* L. and *Erigeron annuus* (L.) Pers. Zeitschrift fur Naturforschung C 65:642–646
Ndiaye D, Diallo M, Sene PD, Ndiaye M, Ndir O (2011) Disseminated histoplasmosis due to *Histoplasma capsulatum* var. *duboisii* in Senegal: A case in HIV-infected patient. J Mycol Med 21(1):60–64
Nelesh PG, Tom MC, Bhavani P, John A (2011) Neglected fungal diseases *in* Sub-Saharan Africa: a call to action. Curr Fungal Infect Rep 5:224–232
Ngameni B, Kuete V, Konga Simo I, Mbaveng AT, Awoussong PK, Patnam R, Roy R, Ngadjui BT (2009) Antibacterial and antifungal activities of the crude extract and compounds from *Dorstenia turbinata* (Moraceae). S Afr J Bot 75:256–261
Ngondi J, Oben J, Minka S (2005) The effect of *Irvingia gabonensis* seeds on body weight and blood lipids of obese subject in Cameroon. Lipids Health Dis 4:12–342
Ngono AN, Biyiti L, Amvam Zollo PH, Bouchet P (2000) Evaluation of antifungal activity of extracts of two cameroonian rutaceae: *Zanthoxylum leprieurii* Guill.et Perr. and *Zanthoxylum xanthoxyloides* Waterm. J Ethnopharmacol 70:335–342
Ngoumfo RM, Jouda JB, Mouafo FT, Komguem J, Mbazoa CD, Shiao TC, Choudhary MI, Laatsch H, Legault J, Pichette A, Roy R (2010) *In vitro* cytotoxic activity of isolated

acridones alkaloids from *Zanthoxylum leprieurii* Guill. et Perr. Bioorg Med Chem 18(10): 3601–3605
Ngounou FN, Manfouo RN, Tapondjou LA, Lontsi D, Kuete V, Penlap V, Etoa FX, Dubois MAL, Sondengam BL (2005) Antimicrobial diterpenoid alkaloids from *Erythrophleum suaveolens* (Guill. & Perr.). Brenan. Bull Chem Soc Ethiop 19:221–226
Noumi E, Yomi A (2001) Medicinal plants used for intestinal diseases in Mbalmayo region, Central Province, Cameroon. Fitoterapia 72:246–254
Noumi E, Dibakto TW (2002) Medicinal plants used for peptic ulcer in the Bangangté region, western Cameroon. Fitoterapia 71:406–512
Noumi E (1984) Les plantes à epices, à condiments et à aromates du Cameroun, These de Doctorat en Sciences biologiques, Universite de Yaounde, Cameroun, p 117
Nweze EI (2010) Dermatophytosis in western Africa: a review. Pak J Biol Sci 13(13):649–656
Nweze EI, Ogbonnaya UL (2011) Oral candida isolates among HIV-infected subjects in Nigeria. J Microbiol Immunol Infect 44(3):172–177
Nwude N, Ibrahim MA (1980) Plants used in traditional veterinary medical practice in Nigeria. J Vet Pharmacol Ther 3:261–273
Odebiyi OO (1978) Preliminary phytochemical and antimicrobial examinations of leaves of *Securidaca longipedunculata*. Nig J Pharm Res 9:29–30
Odhiambo JA, Siboe GM, Lukhoba CW, Saiffudin FD (2010) Antifungal activity of crude extracts of *Gladiolus dale*nii van geel (Iridaceae). Afr J Tradit Complement Altern Med 7:53–58
Ofori-Kwakye K, Kwapong AA, Adu F (2009) Antimicrobial activity of extracts and topical products of the stem bark of *Spathodea campanulata* for wound healing. Afr J Tradit Complement Altern Med 6:168–174
Ogunwande IA, Walker TM, Bansal A, Setzer WN, Essien EE (2010) Essential oil constituents and biological activities of *Peristrophe bicalyculata* and *Borreria verticillata*. Natur Prod Commun 5:1815–1818
Okemo PO, Harsh PB, Jorge MV (2003) In vitro activities of *Maesa lanceolata* extracts against fungal plant pathogens. Fitoterapia 74:312–316
Okigbo RN, Mmeka EC (2008) Antimicrobial effects of three tropical plant extracts on *staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. Afr J Tradit Complement Altern Med 5:226–229
Okonkwo NJ and Umeanaeto PU (2010) Prevalence of vaginal candidiasis among pregnant women in Nnewi Town of Anambra State, Nigeria http://ajol.info/index.php/afrrev/index. Accessed on Dec 10 2011
Okpekon T, Yolou S, Gleye C, Roblot F, Loiseau P, Bories C, Grellier P, Frappier F, Laurens A, Hocquemiller R (2004) Antiparasitic activities of medicinal plant used in Ivory Coast. J Ethnopharmacol 90:91–97
Okunji CO, Iwu MM (1991) Molluscidal activity of *Garcinia kola* biflavanones. Fitoterapia 62:74–76
Oliver B (1960) Medicinal plants in Nigeria. Nigerian College of Arts, Science and Technology, Lagos
Orie NN, Ekon EU (1993) The bronchodilator effects of *Garcinia kola*. East Afr Med J 70:143–145
Osadebe PO, Akabogu IC (2006) Antimicrobial activity of *Loranthus micranthus* harvested from kola nut tree. Fitoterapia 77:54–56
Palgrave KC, Drummond RB (1977) Trees of Southern Africa. C. Struik, Cape Town
Pankhurst A (2000) Awliyaw: the large stand oldest tree in Ethiopia. http://www.addistribune.com/Archives/2000/02/18-02-00/Awliy.htm. Accessed 9 Nov 2011
Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, Chiller TM (2009) Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. AIDS 23:525–530
Parmar VS, Jain SC, Bisht KS, Jain R, Taneja P, Jha A, Tyagi OD, Prasad AK, Wengel J, Olsen CE, Bol PM (1997) Phytochemistry of the genus *Piper*. Phytochemistry 46(4):591–673

Patra A, Jha S, Murthy PN, Manik and Sharone A (2010) Isolation and characterization of stigmast-5-en-3β-ol (β-sitosterol) from the leaves of *Hygrophila spinosa* T. Anders. Int J Pharma Sci Res 1:95–100

Pegel KH, Rogers CB (1968) Constituents of *Bridelia micrantha*. Phytochemistry 7(4):655–656

Pell SK (2004) Molecular systematics of the cashew family (Anacardiaceae). Doctoral dissertation, Louisiana State University, Baton Rouge

Perdue REJ, Tsichritzis F, Jakupovi J (1993) Prevernocistifolides from *Vernonia galamensis*. Phytochemistry 34(4):1075–1077

Pesewu GA, Cutler RR, Humber DP (2008) Antibacterial activity of plants used in traditional medicines of Ghana with particular reference to MRSA. J Ethnopharmacol 116:102–111

Pieme CA, Dzoyem JP, Penlap V, Ngongang J, Etoa FX (2008) *In vitro* antimicrobial activity of seventeen crude extracts from ten Cameroonian medicinal plants. J Biol Sci 8(5):902–907

Pienaar ED, Young T, Holmes H (2010) Interventions for the prevention and management of oropharyngeal candidiasis associated with HIV infection in adults and children. Cochrane Database Systematic Reviews http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD003940.pub3/pdf. Accessed on Dec 12 2011

Pooley E, (1993) Trees of Natal, Zululand and Transkei. Natal Flora Publications Trust, Durban

Ragasa CY, Ha HK, Hasika M, Maridable JB, Gaspillo PD, Rideout JA (2008) Antimicrobial and cytotoxic terpenoids from *Cymbopogon citratus* Stapf. Philippine Sci 45:111–122

Rastogi S, Pandey MM, Rawat KSA (2011) An ethnomedicinal, phytochemical and pharmacological profile of *Desmodium gangeticum* (L.) DC. and *Desmodium adscendens* (Sw.) DC. J Ethnopharmacol 136:283–296

Rayne S, Mazza G (2007) Biological activities of extracts from sumac (Rhus spp.): a review. Plant Foods Hum Nutr 62:165–175

Ready PM, Adams PR, Robertus DJ (1984) Dodecandrin, a new ribosome-inhibiting protein from *Phytolacca dodecandra*. Biochim Biophys Acta 791(3):314–319

Rolfsen WNA, Olaniyi AA, Hylands JP (1980) New tertiary alkaloids of *Strychnos decussate*. J Nat Prod 43(1):97–102

Ronsted N, Franzyk H, Molgaard P, Jaroszewski JW, Jensen SR (2003) Chemotaxonomy and evolution of Plantago L. Plant Syst Evol 242:63–82

Roosita K, Kusharto CM, Sekiyama M, Fachrurozi Y, Ohtsuka R (2008) Medicinal plants used by the villagers of a Sundanese community in West Java, Indonesia. J Ethnopharmacol 115:72–81

Rukunga GM, Muregi FW, Tolo FM, Omar SA, Mwitari P, Muthaura CN, Omlin F, Lwande W, Hassanali A, Githure J, Iraqi FW, Mungai GM, Kraus W, Kofi-Tsekpo WM (2007) The antiplasmodial activity of spermine alkaloids isolated from *Albizia gummifera*. Fitoterapia 78:455–459

Sahakitpichan P, Disadee W, Ruchirawat S, Kanchanapoom T (2010) L-(-)-(N-trans-cinnamoyl)-arginine, an acylamino acid from *Glinus oppositifolius* (L.) Aug. DC. Molecules 15:6186–6192

Samie A, Tambani T, Harshfield E, Green E, Ramalivhana JN, Bessong PO (2010) Antifungal activities of selected Venda medicinal plant *against Candida albicans, Candida krusei* and *Cryptococcus neoformans* isolated from South Africa AIDS patients. Afr J Biotechnol 9:2965–2976

Sandberg F, Premila PI, Hesham RES (2005) A Swedish collection of medicinal plants from Cameroon. J Ethnopharmacol 102:336–343

Sangare A, Yoboue P, Ahogo C, Ecra E, Kaloga M, Gbery I, Kanga JM (2008) Disseminated cutaneous histoplasmosis due to *Histoplasma capsulatum* var. *duboisii* associated with AIDS: a case report in Abidjan, Côte d'Ivoire. Bulletin de la Société de Pathologie Exotique 101(1):5–7

Sanz-Peláez O, Carranza RC, Gutiérrez VIR, Pérez-Arellano JL (2007) *Histoplasma capsulatum* infection in African sub-Saharian population. Med Clin 129(8):319

Sarker SD, Bartholomew B, Nash RJ (2000) Alkaloids from *Balanites aegyptiaca*. Fitoterapia 71:328–330

Saxton JE (1987) Recent progress in the chemistry of indole alkaloids and mould metabolites. Nat Prod Rep 4:591–637
Schmidt B, Ribnicky DM, Poulev A, Logendra S, Cefalu WT, Raskin I (2008) A natural history of botanical therapeutics. Metabolism 57(7 Suppl 1):S3–S9
Seigler SD, Butterfield SC, Dunn EJ, Conn EE (1975) Dihydroacacipetalin–a new cyanogenic glucoside from *Acacia sieberiana* var. Woodii. Phytochemistry 14(5–6):1419–1420
Sequeira BJ, Vital MJ, Pohlit AM, Pararols IC, Caúper GS (2009) Antibacterial and antifungal activity of extracts and exudates of the Amazonian medicinal tree *Himatanthus articulatus* (Vahl) Woodson (common name: sucuba). Mem Inst Oswaldo Cruz 104:659–661
Shealy CN (1998) The illustrated encyclopaedia of healing remedies. Element Books, Australia
Shrum JP, Millikan LE, Bataineh O (1994) Superficial fungal infections in the tropics. Dermatol Clinic 12:687–693
Shuaibu NM, Wuyep TAP, Yanagi T, Hirayama K, Ichinose A, Tanaka T, Kouno I (2008) Trypanocidal activity of extracts and compounds from the stem bark of *Anogeissus leiocarpus* and *Terminalia avicennoides*. Parasitol Res 102:697–703
Simon G, Dewelle J, Nacoulma O, Guissou P, Kiss R, Daloze D, Braekman JC (2003) Cytotoxic pentacyclic triterpenes from *Combretum nigricans*. Fitoterapia 74(4):339–344
Sindambiwe JB, Aliou MB, Tess DB, Pieters L, Van den Heuvel H, Magda C, Van Den Berghe AD, Vlietinck JA (1996) Triterpenoid saponins from *Maesa lanceolata*. Phytochemistry 41:269–277
Sofowora A (1980) The present status of knowledge of the plants used in traditional medicine in western Africa: a medical approach and a chemical evaluation. J Ethnopharmacol 2:109–118
Sofowora EA, Isaac-Sodeye WA, Ogunkoya LO (1975) Isolation and characterisation of an antisickling agent from *Fagara zanthoxyloides* root. Lloydia 38:169–171
Ssegawa P, Kasenene JM (2007) Medicinal plant diversity and uses in the Sango Bay area, Southern Uganda. J Ethnopharmacol 113:521–540
Staubmann R, Schubert-Zsilavecz M, Hiermann A, Kartnig T (1999) A complex of 5-hydroxy-pyrrolidin-2-one and pyrimidine-2,4-dione isolated from *Jatropha curcas*. Phytochemistry 50:337–338
Steenkamp V, Fernandes AC, Van Rensburg CEJ (2007) Screening of Venda medicinal plants for antifungal activity against *Candida albicans*. S Afr J Bot 73:256–258
Stevens PF (2001) Angiosperm phylogeny. Website Version 9, June 2008, http://www.mobot.org/mobot/research/apweb/welcome.html
Stevensen CJ (1998) Aromatherapy in dermatology. Clin Dermatol 16:689–694
Steyn PS, Van Den Heever JP, Vosloo HCM, Ackerman LGJ (1998) Biologically active substances from *Zanthoxylum capense* (thumb.) Harv. S Afr J Sci 94:391–393
Sudhakar M, Rao CV, Rao PM, Raju DB, Venkateswarlu Y (2006) Antimicrobial activity of *Caesalpinia pulcherrima, Euphorbia hirta* and *Asystasia gangeticum*. Fitoterapia 77:378–380
Suleiman MM, McGaw LJ, Naidoo V, Eloff JN (2010) Evaluation of several tree species for activity against the animal fungal. S Afr J Bot 76:64–71
Sy GY, Nongonierma RB, Ngewou PW, Mengata DE, Dieye AM, Cisse A, Faya B (2005) Healing activity of methanolic extract of the barks of *Spathodea campanulata* beauv (bignoniaceae) in rat experimental burn model. Dakar Med 50:77–81
Tabanca N, Demirci B, Gürbüz I, Demirci F, Becnel JJ, Wedge DE, Başer KH (2011) Essential oil composition of five collections of *Achillea biebersteinii* from central Turkey and their antifungal and insecticidal activity. Nat Prod Commun 6:701–706
Tabuti JRS, Lye KA, Dhillion SS (2003) Traditional herbal drugs of Bulamogi, Uganda: plants, use and administration. J Ethnopharmacol 88:19–44
Takeda T, Narukawa Y, Hada N (1999) Studies on the constituents of *Leonotis nepetaefolia*. Chem Pharm Bull 47(2):284–286
Taniguchi M, Chapya A, Kubo I, Nakanishi K (1978) Screening of East African plants for antimicrobial activity. Chem Pharm Bull 26:2910–2913

Tatsadjieu LN, Essia Ngang JJ, Ngassoum MB, Etoa FX (2003) Antibacterial and antifungal activity of *Xylopia aethiopica*, *Monodora myristica*, *Zanthoxylum xanthoxyloïdes* and *Zanthoxylum leprieurii* from Cameroon. Fitoterapia 74:469–472

Teinkela MJE, Ngouela S, Assob NJC, Beng VP, Rohmer M, Tsamo E (2010) In vitro antimicrobial activity of extracts and compounds of some selected medicinal plants from Cameroon. J Ethnopharmacol 128:476–481

Teke GN, Kuiate JR, Kuete V, Teponno RB, Tapondjou LA, Tane P, Giacinti G, Vilarem G (2011) Bio-guided isolation of potential antimicrobial and antioxidant agents from the stem bark of *Trilepisium madagascariense*. S Afr J Bot 77:319–327

Thembo KM, Vismer HF, Nyazema NZ, Gelderblom WC, Katerere DR (2010) Antifungal activity of four weedy plant extracts against selected mycotoxigenic fungi. J Appl Microbiol 109:1479–1486

Tih A, Martin MT, Tih RG, Vuidepot I, Sondengam BL, Bodo B (1992) Lophiroflavans B and C, tetraflavonoids of *Lophira alata*. Phytochemistry 31(10):3595–3599

Tomczykowa M, Tomczyk M, Jakoniuk P, Tryniszewska E (2008) Antimicrobial and antifungal activities of the extracts and essential oils of *Bidens tripartita*. Folia Histochem Cytobiol 46:389–393

Tra Bi FH, Koné MW, Kouamé NF (2008) Antifungal activity of *Erigeron floribundus* (Asteraceae) from Côte d'Ivoire, West Africa. Trop J Pharm Res 7:975–979

Traeder C, Kowoll S, Arastéh K (2008) Candida infection in HIV positive patients 1985–2007. Mycoses 51(Suppl 2):58–61

Tsopmo A, Tene M, Kamnaing P, Ayafor JF, Sterner A (1999) A new diels-alder type adduct flavonoids from *D. barteri*. J Nat Prod 62:1432–1434

Vandeputte P, Selene F, Coste AT (2012) Antifungal resistance and new strategies to control fungal infections. Int J Microbiol. doi:10.1155/2012/713687

Van den Berg AJJ, Horsten SFAJ, Kettenes-van den Bosch JJ, Kroes BH, Beukelman CJ, Leeflang BR, Labadie RP (1995) Curcacycline A a novel cyclic octapeptide isolated from the latex of *Jatropha curcas* L. FEBS Lett 358:215–218

Van Wyk BE, Van Oudtshoorn B, Gericke N (1997) Medicinal plants of South Africa, 1st edn. Eriza, Pretoria, South Africa

VanVuuren SF, Kamatou GPP, Viljoen AM (2010) Volatile composition and antimicrobial activity of twenty commercial Frankincense essential oil samples. S Afr J Bot 76:686–691

Veale DJH, Furman KI, Oliver DW (1992) South African traditional herbal medicines used during pregnancy and child birth. J Ethnopharmacol 36:185–191

Vismer HF, Hull PR (1997) Prevalence, epidemiology and geographical distribution of *Sporothrix schenckii* infections in Gauteng, South Africa. Mycopathologia 137:137–143

Vital PG, Rivera WL (2011) Antimicrobial activity, cytotoxicity, and phytochemical screening of *Voacanga globosa* (blanco) Merr. leaf extract (apocynaceae). Asian Pac J Trop Med 4:824–828

Vlietinck AJ, Van Hoof L, Totte J, Lasure A, Vanden Berghe D, Rwangabo PC, Mvukiyumwami J (1995) Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties. J Ethnopharmacol 46:31–47

Walker AR, Sillans R (1961) Les plantes utiles du Gabon. Paul Lechevalier, Paris

Walker AR (1953) Usages Pharmaceutiques des plantes spontanus du Gabon. Insitut d'Etudes Centra Fracaines 9:13–26

Wanyonyi WA, Chhabra CS, Mkoji G, Eilert U, Njue MW (2002) Bioactive steroidal alkaloid glycosides from *Solanum aculeastrum*. Phytochemistry 59(1):79–84

Watt JM, Breyer-Brandwijk MG (1962) The medicinal and poisonous plants of Southern and Eastern Africa, 2nd edn. Livingstone, London

Wootton S (2005) Aromatherapy and natural health. GEF abbri Ltd, London

Yagi SM, El Tigani S, Adam SEI (1998) Toxicity of *Senna obtusifolia* fresh and fermented leaves (kawal), *Senna alata* leaves and some products from *Senna alata* on rats. Phytother Res 12:324–330

Yenesew A, Induli M, Derese S, Midiwo OJ, Heydenreich M, Peter GM, Akala H, Wangui J, Liyala P, Waters CN (2004) Anti-plasmodial flavonoids from the stem bark of *Erythrina abyssinica*. Phytochemistry 65:3029–3032

Yi T, Wen J, Golan-Goldhirsh A, Parfitt DE (2008) Phylogenetics and reticulate evolution in pistacia (anacardiaceae). Am J Bot 95:241–251

Yoshida M, Fuchigami M, Nagao T, Okabe H, Matsunaga K, Takata J, Karube Y, Tsuchihashi R, Kinjo J, Mihashi K, Fujioka T (2005) Antiproliferative constituents from umbelliferae plants VII. Active triterpenes and rosmarinic acid from *Centella asiatica*. Biol Pharm Bull 28:173–175

Yu GD (1982) Medicinal plants used for abortion and childbirth in Eastern Africa. Chung Yao T'ung Pao 7:6–7

Zhao BX, Wang Y, Zhang DM, Jiang RW, Wang GC, Shi JM, Huang XJ, Chen WM, Che CT, Ye WC (2011) Flueggines A and B, two new dimeric indolizidine alkaloids from *Flueggea virosa*. Org Lett 13(15):3888–3891

Zheng CJ, Yoo JS, Lee TG, Cho HY, Kim YH, Kim WG (2005) Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids. FEBS Lett 579:5157–5162

Part II
A. Antifungal Activities of Plants and Other Natural Products

Chapter 5
Natural Products as Potential Resources for Antifungal Substances: A Survey

Mahmud Tareq Hassan Khan

Abstract Due to many reasons, potent and new antifungal agents are still required, especially for the large populations of immunocompromised patients; it is lifesaving to protect them from systemic infections. Scientists around the globe are hunting for new and potent antifungal molecules. Nature as well as ethnomedicine thought to be a very good source of novel molecules not only against fungal infections, but also against many other clinical complications, like cancers and so on. This chapter puts some emphasis on and discusses about the natural products as source of antifungal agents.

5.1 Introduction

All the way through the history and across the globe, the plant kingdom has provided a variety of medicines. In the modern era, plants have been a source of analgesics, anti-inflammatory, anticancer molecules, substances for asthma, antiarrhythmic agents, and antihypertensive. Plants with antimicrobial activity are also known to be numerous, yet prior to a decade ago, minimal research had been conducted in the area of antifungal medicinal plants (Webster et al. 2008).

The requirements for new antifungal agents remain, driven by devious infections in immunocompromised patients and by the development of resistance to existing agents (Jacob and Walker 2005). The incidence and prevalence of invasive fungal infections have dramatically increased in recent years due to a remarkable elevation in the number of immunocompromised patients and those hospitalized in intensive care units (ICUs) (Arendrup et al. 2005; Enoch et al. 2006; Espinel-Ingroff 2009). In addition, the mortality and morbidity of these

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infections are quite substantial. The most common fungal pathogens continue to be the species of *Candida* and *Aspergillus* (Arendrup et al. 2005; Lass-Florl et al. 2005; Pfaller and Diekema 2007; Espinel-Ingroff 2009).

Although the current antifungal therapies for invasive fungal infections have been significantly improved, the outcome is still unsatisfactory because of the limited target sites available for antifungal drug design, the development of resistance to current antifungals, and the problems of early diagnosis. Recent advances in the development of new antifungals, although still in the investigational stages, offer some new hope of improving the future of antifungal therapy (Vazquez 2007; Zhai and Lin 2011).

Several studies investigating the antifungal susceptibility of clinical strains of *Candida* species implied emergence of new resistant strains (Resende and Resende 1999). Disseminated cryptococcosis affects a more limited percentage of patients (6–8 %), yet is still a serious life-threatening condition (Chen et al. 1996; Fostel and Lartey 2000; Fortes et al. 2008).

Natural products propose virtually an unlimited source of novel and diverse bioactive compounds and not only serve as a reservoir for new potential drugs and drug prototypes, but also for probes of fungal biology (Jacob and Walker 2005).

This chapter highlights a critical survey on scientific reports on antifungal substances identified in plants and natural products.

5.2 Antifungal Agents: Present Status

In the United States, only limited numbers of antifungal drugs belonging to three major classes, that is, polyenes, pyrimidines, and azoles, are currently approved by the Food and Drug Administration (FDA) for the therapy of systemic fungal infections. Some drugs belonging to other classes are also approved as topical antifungal drugs (Dismukes 2012). The use of standard antifungal therapies encounters many challenges due to their toxicities, limited routes of administration, low efficacy rates, and drug resistance. Various new antifungal agents have now provided good alternatives for overcoming the limitations of current treatments (Gupta and Tomas 2003). The azole antifungal agents represented a major advance in the management of systemic fungal infections (Dismukes 2012) over the past two decades. In contrast to miconazole, the first azole drug to be approved, the three oral azoles, that is, ketoconazole, itraconazole and fluconazole, have been frequently used as alternatives to amphotericin B (Hoesley and Dismukes 1997; Kauffman and Carver 1997a, b). These antifungals received major attention due to broad spectrum of activity against common fungal pathogens from pathogenic yeasts, that is, *Candida* species and *Cryptococcus neoformans* to dimorphic true pathogens (*Blastomyces dermatitidis, Histoplasma capsulatum, Coccidioides immitis, Paracoccidioides brasiliensis*), *Sporothrix schenckii*, and *Aspergillus* species (Dismukes 2012). Among the oral azoles, fluconazole is a drug of choice due to possessing high bioavailability, high water solubility, low levels of protein

binding, wide distribution into body tissues and fluids, especially cerebrospinal fluid and urine, and long half-life (Grant and Clissold 1989, 1990; Goa and Barradell 1995). In addition, fluconazole and itraconazole are better tolerated and more effective than ketoconazole (Dismukes 2012).

Due to the escalation of HIV, opportunistic fungal pathogens have become a common source of morbidity and mortality (Garbino et al. 2001). Consequently, the frequency of opportunistic infections, including fungal infections, has also increased (Bathoorn et al. 2013). Therefore, antifungal therapy is playing a greater role in health care, and the screening of traditional plants in search of novel antifungals is now more frequently performed (Motsei et al. 2003).

5.3 Traditional Medicine Against Fungal Infections

Tendency toward use of herbal drugs has dramatically increased due to a large number of drawbacks in synthetic antifungal drugs (Meena et al. 2009). Recent interests in traditional pharmacopoeias have meant that scientists around the globe are concerned not only with determining the scientific rationale for the usage of plants, but also with the discovery of novel compounds with pharmaceutical values (Fennell et al. 2004). Instead of depending on "trial and error," as in random screening procedures, knowledge from ethnomedicinal or traditional medicines helps researchers to target plants that may be medicinally beneficial (Cox and Balick 1994). An estimated number of 122 drugs from 94 plant species have been discovered through ethnobotanical studies (Fabricant and Farnsworth 2001).

Several plants are used by traditional healers in the treatment for oral candidiasis (Fennell et al. 2004). Extracts were tested for antifungal activity against *Candida albicans* in a microdilution assay (Fennell et al. 2004). This assay is widely used and is a well-recognized technique in the screening of plants for antimicrobial activity (Espinell-Ingroff and Pfaller 1995). Aqueous extracts from *Tulbaghia violacea* Harv., *Allium sativum* L. (both are from the Family Alliaceae), *Polygala myrtifolia* L. (Family Polygalaceae), and *Glycyrrhiza glabra* L. (Family Fabaceae) exhibited activity with minimal inhibitory concentration (MIC) values between 6.25 and 12.5 mg/ml (Motsei et al. 2003).

5.4 Plant and Plant Extracts Against Fungi and Fungal Infections: Examples

Johann et al. (2007) reported the antifungal properties of extracts from eight Brazilian plants traditionally used in popular Brazilian medicines against five clinically relevant *Candida* species, *C. neoformans*, and *S. schenckii*. Their results demonstrated that all the extracts possessed antifungal activities. The ethanolic extract from the leaves from *Schinus terebinthifolius* exhibited potential antifungal

activity against both the fungi. Authors also reported preliminary phytochemical analyses of the extract of *S. terebinthifolius* which exhibited the presence of biologically active compounds, like saponins, flavonoids, triterpenes, steroids, and tannins.

During 2008, Bansod and Rai screened and reported the antifungal activities of essential oils extracted from fifteen medicinal plants against *Aspergillus fumigatus* and *A. niger* (Bansod and Rai 2008). Utilizing agar diffusion method, authors measured the minimum inhibitory concentrations (MICs) of oils against the fungi. Their results exhibited that the maximum antimycotic activity was demonstrated by oils from *Cymbopogon martini, Eucalyptus globulus*, and *Cinnamomum zylenicum* when compared to control, followed by *Cymbopogon citratus* which showed activity similar to control (miconazole nitrate). The oils from *Mentha spicata, Azadirachta indica, Eugenia caryophyllata, Withania somnifera*, and *Zingiber officinale* showed moderate activities, and oils from *Cuminum cyminum, Allium sativum, Ocimum sanctum, Trachyspermum copticum, Foeniculum vulgare*, and *Elettaria cardamomum* exhibited comparatively lower antifungal activities (Bansod and Rai 2008).

The total extracts from the aerial parts of *Senecio aegyptius* var. *discoideus* Boiss (Family Asteraceae) exhibited very good antifungal activity against *C. albicans* and *Saccharomyces cerevisiae*. A new eremophilane sesquiterpene, 1-beta-hydroxy-8-oxoeremophila-7,9-dien-12-oic acid, in addition to two known flavonol glycosides, rutin and quercetin-3-O-glucoside-7-O-rutinoside, has been isolated from the ethyl acetate fraction obtained from the aqueous alcoholic extract of the plant (Hassan et al. 2012).

5.5 Antifungal Compounds from Natural Resources

Medicinal plants have been a source of wide variety of biologically active compounds for many centuries and used extensively as crude material or as pure compounds for treating various disease conditions (Arif et al. 2009, 2011). There are number of compounds isolated and reported from plants to be moderate to potent antifungal.

Phenolic acid derivatives—crassinervic acid (structure shown in Fig. 5.1), aduncumene, hostmaniane and gaudichaudianic acid—have been isolated and reported from *Piper crassinervium*, *Piper aduncum*, *Piper hostmannianum*, and *Piper gaudichaudianum*, respectively, reported to show fungitoxic properties (Lago et al. 2004). Eriosemaones A–D (structures are shown in Fig. 5.1) exhibited good antifungal activities (Ma et al. 1995). Constituents from *Croton hutchinsonianus*, and pinosylvin (structure shown in Fig. 5.1), a constituent of pine, showed growth inhibitory activity against *C. albicans* and *S. cerevisiae* (Athikomkulchai et al. 2006).

Flavonoids isolated from the stem bark of *Erythrina burtii* were reported for antifungal activity (Yenesew et al. 2005). The compound 4-methoxy-5,7-

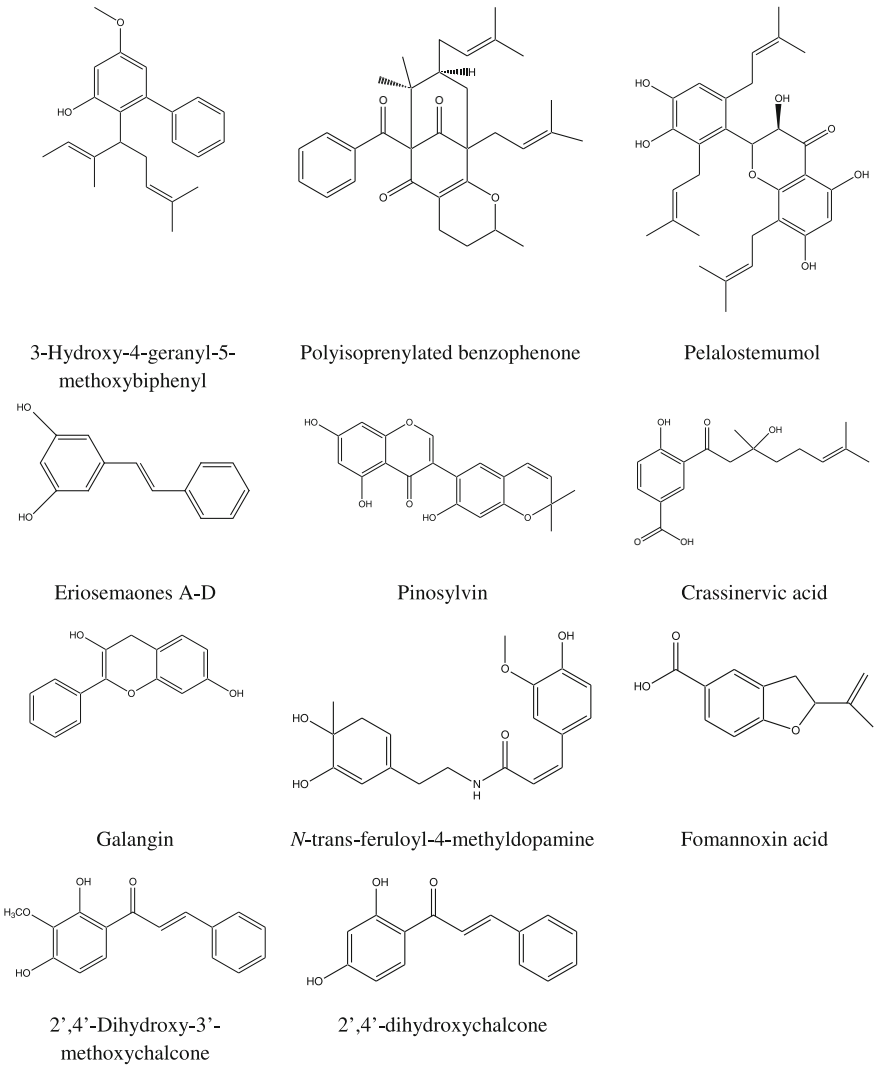

Fig. 5.1 Molecular structures of some antifungal compounds reported in literatures (Hufford et al. 1993; Ma et al. 1995; Afolayan and Meyer 1997; Lago et al. 2004; Athikomkulchai et al. 2006; Rahman et al. 2007; Svetaz et al. 2007; Arif et al. 2009, 2011)

dihydroxyflavone 6-C-glucoside (isocytisoside) isolated from the leaves and stems of *Aquilegia vulgaris* exhibited activities against *A. niger* (Bylka et al. 2004).

The compound petalostemumol (structure is shown in Fig. 5.1) from *Petalostemum purpureum* showed strong antifungal activity against many pathogenic fungi (Hufford et al. 1993). Galangin (structure shown in Fig. 5.1), derived from the perennial herb *Helichrysum aureonitens*, exhibited antifungal activities against wide range of fungi (Afolayan and Meyer 1997).

Some of the representative compounds with antifungal activities and their structures are shown in Fig. 5.1.

The compound *N*-trans-feruloyl-4-methyldopamine (structure shown in Fig. 5.1) has been isolated from *Achyranthes ferruginea* and was reported to be active against a broad range of fungi (Rahman et al. 2007).

The antifungal chromenes, methyl 2,2-dimethyl-2H-1-chromene-6-carboxylate and methyl 2,2-dimethyl-8-(3′-methyl-2′-butenyl)-2H-1-chromene-6-carboxylate, have been isolated and reported from the leaves of *Piper aduncum* (Tan et al. 1996a, b).

The compounds iridodial *β*-monoenol acetate, from *Nepeta leucophylla*, and actinidine from *N. clarkei* were found to be endowed with antifungal activities (Saleh et al. 2006).

Anofinic acid and fomannoxin acid (structure shown in Fig. 5.1) from *Gentiana algida* were found to be active against fungi (Arif et al. 2009, 2011).

The antifungal activity of *Artemisia herba-alba* was found to be associated with two major volatile compounds as carvone and piperitone (Vollekova et al. 2003).

The compounds 2′,4′-dihydroxy-3′-methoxychalcone and 2′,4′-dihydroxychalcone (structures shown in Fig. 5.1) have been isolated from the dichloromethane fraction of *Zuccagnia punctata* found to have antifungal properties (Svetaz et al. 2007).

5.6 Conclusion

In the concluding remarks, Fennel et al. (2004) in their review mentioned that in vitro screening programs, using the ethnomedicinal approaches, are important in validating the traditional use of herbal remedies and for providing leads in the search for new active principles (Fennell et al. 2004). Whereas, activity identified by in vitro tests do not necessarily confirm that plant extracts are effective medicines, nor a suitable candidate for drug development, though it does provide basic understanding of a plant's efficacy and, in some cases toxicity, in a traditional herbal remedy (Fennell et al. 2004).

Most of the numerous compounds with proven antifungal activity are at the in vitro or animal models of efficacy stages. Therefore, further investigation should be carried out to realize the true efficacy of these compounds in clinic (Espinel-Ingroff 2009). According to Denning and Hope (2010), the critical characteristic for a new antifungal drug is oral bioavailability in treatment for invasive aspergillosis, invasive candidiasis, cryptococcal meningitis, and mucosal and urinary *Candida* infections. They implied that beyond antifungal drug resistance as a major problem, early mycological diagnosis and improvements in underlying risk estimation enable the clinicians to improve outcomes.

Among seven classes of antifungal agents currently available only three classes including polyenes, azoles, and echinocandins are suitable for treatment of systemic infections. "None match all the characteristics of an ideal agent, the Holy

Grail of antifungal therapy" (Chapman et al. 2008). Academia and industry need to collaborate in the search for new lead antifungal molecules using traditional and the new pharmacogenomics methods. Enhancing efficacy and reducing toxicity of the currently available antifungal agents may also consider as another important aspect of the study (Chapman et al. 2008).

References

Afolayan AJ, Meyer JJ (1997) The antimicrobial activity of 3,5,7-trihydroxyflavone isolated from the shoots of *Helichrysum aureonitens*. J Ethnopharmacol 57(3):177–181

Arendrup MC, Fuursted K, Gahrn-Hansen B, Jensen IM, Knudsen JD, Lundgren B, Schonheydar HC, Tvede M (2005) Seminational surveillance of fungemia in Denmark: notably high rates of fungemia and numbers of isolates with reduced azole susceptibility. J Clin Microbiol 43(9):4434–4440

Arif T, Bhosale JD, Kumar N, Mandal TK, Bendre RS, Lavekar GS, Dabur R (2009) Natural products: antifungal agents derived from plants. J Asian Nat Prod Res 11(7):621–638

Arif T, Mandal TK, Dabur R (2011) Natural products: anti-fungal agents derived from plants. In: Tiwari VK, Mishra BB (eds) Opportunity, challenge and scope of natural products in medicinal chemistry. Research Signpost, Kerala, pp 283–311

Athikomkulchai S, Prawat H, Thasana N, Ruangrungsi N, Ruchirawat S (2006) COX-1, COX-2 inhibitors and antifungal agents from *Croton hutchinsonianus*. Chem Pharm Bull 54(2): 262–264 (Tokyo)

Bansod S, Rai M (2008) Antifungal activity of essential oils from Indian medicinal plants against human pathogenic *Aspergillus fumigatus* and *A. niger*. World J Med Sci 3(2):81–88

Bathoorn E, Escobar Salazar N, Sepehrkhouy S, Meijer M, de Cock H, Haas PJ (2013) Involvement of the opportunistic pathogen *Aspergillus tubingensis* in osteomyelitis of the maxillary bone: a case report. BMC Infect Dis 1(13):59. doi:10.1186/1471-2334-13-59

Bylka W, Szaufer-Hajdrych M, Mattawska I, Goślińska O (2004) Antimicrobial activity of isocytisoside and extracts of *Aquilegia vulgaris* L. Lett Appl Microbiol 39(1):93–97

Chapman SW, Sullivan DC, Cleary JD (2008) In search of the holy grail of antifungal therapy. Trans Am Clin Climatol Assoc 119:197–215

Chen LC, Blank ES, Casadevall A (1996) Extracellular proteinase activity of *Cryptococcus neoformans*. Clin Diagn Lab Immunol 3(5):570–574

Cox PA, Balick MJ (1994) The ethnobotanical approach to drug discovery. Sci Am 270(6):82–87

Denning DW, Hope WW (2010) Therapy for fungal diseases: opportunities and priorities. Trends Microbiol 18(5):195–204

Dismukes WE (2012) Introduction to antifungal drugs. Guidelines for antibiotic therapy at the hospital of the University of Pennsylvania. Retrieved 13 July 2012, from http://www.uphs.upenn.edu/bugdrug/

Enoch DA, Ludlam HA, Brown NM (2006) Invasive fungal infections: a review of epidemiology and management options. J Med Microbiol 55(Pt 7):809–818

Espinel-Ingroff A (2009) Novel antifungal agents, targets or therapeutic strategies for the treatment of invasive fungal diseases: a review of the literature (2005–2009). Rev Iberoam Micol 26(1):15–22

Espinell-IngroffAPfaller MA (1995) Antifungal agents and susceptibility testing. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH (eds) Manual of clinical microbiology. ASM Press, Washington

Fabricant DS, Farnsworth NR (2001) The value of plants used in traditional medicine for drug discovery. Environ Health Prospect 109(1):69–75

Fennell CW, Lindsey KL, McGaw LJ, Sparg SG, Stafford GI, Elgorashi EE, Grace OM, van Staden J (2004) Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicology. J Ethnopharmacol 94(2–3):205–217

Fortes TO, Alviano DS, Tupinambá G, Padrón TS, Antoniolli AR, Alviano CS, Seldin L (2008) Production of an antimicrobial substance against *Cryptococcus neoformans* by *Paenibacillus brasilensis* Sa3 isolated from the rhizosphere of Kalanchoe brasiliensis. Microbiol Res 163(2):200–207

Fostel JM, Lartey PA (2000) Emerging novel antifungal agents. Drug Discov Today 5(1):25–32

Garbino J, Kolarova L, Lew D, Hirschel B, Rohner P (2001) Fungemia in HIV-infected patients: a 12-year study in a tertiary care hospital. AIDS Patient Care STDS 15(8):407–410

Goa KL, Barradell LB (1995) Fluconazole: an update of its pharmacodynamic and pharmacokinetic properties and therapeutic use in major superficial and systemic mycoses in immunocompromised patients. Drugs 50(4):658–690

Grant SM, Clissold SP (1989) Itraconazole: a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in superficial and systemic mycoses. Drugs 37(3): 310–344

Grant SM, Clissold SP (1990) Fluconazole: a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in superficial and systemic mycoses. Drugs 39(6):877–916

Gupta AK, Tomas E (2003) New antifungal agents. Dermatol Clin 21(3):565–576

Hassan W, Al-Gendy A, Al-Youssef H, El-Shazely A (2012) Chemical constituents and biological activities of *Senecio aegyptius* var. *discoideus* Boiss. Z Naturforsch [C] 67(3–4):144–150

Hoesley C, Dismukes WE (1997) Overview of oral azole drugs as systemic antifungal therapy. Semin Resp Crit Care Med 18:301–309

Hufford CD, Jia Y, Croom EM Jr, Muhammed I, Okunade AL, Clark AM, Rogers RD (1993) Antimicrobial compounds from *Petalostemum purpureum*. J Nat Prod 56(11):1878–1889

Jacob MR, Walker LA (2005) Natural products and antifungal drug discovery. Methods Mol Med 118:83–109

Johann S, Pizzolatti MG, Donnici CL, Resende MA (2007) Antifungal properties of plants used in Brazilian traditional medicine against clinically relevant fungal pathogens. Braz J Microbiol 38:632–637

Kauffman CA, Carver PL (1997a) Antifungal agents in the 1990s. Current status and future developments. Drugs 53(4):539–549

Kauffman CA, Carver PL (1997b) Use of azoles for systemic antifungal therapy. Adv Pharmacol 39:143–189

Lago JH, Ramos CS, Casanova DC, Morandim Ade A, Bergamo DC, Cavalheiro AJ, Bolzanivda S, Furlan M, Guimarães EF, Young MC, Kato MJ (2004) Benzoic acid derivatives from Piper species and their fungitoxic activity against *Cladosporium cladosporioides* and *C. sphaerospermum*. J Nat Prod 67(11):1783–1788

Lass-Florl C, Mayr A, Mayr A, Gastl G, Bonatti H, Freund M, Kropshofer G, Dierich MP, Nachbaur D (2005) Epidemiology and outcome of infections due to *Aspergillus terreus*: 10-year single centre experience. Br J Haematol 131(2):201–217

Ma WG et al (1995) Polyphenols from *Eriosema tuberosum*. Phytochemistry 39(5):1049–1061

Meena AK, Niranjan US, Rao MM, Padhi MM, Babu R (2009) Review on antifungal activities of Ayurvedic medicinal plants. Drug Invention Today 2(2):146–148

Motsei ML, Lindsey KL, van Staden J, Jäger AK (2003) Screening of traditionally used South African plants for antifungal activity against *Candida albicans*. J Ethnopharmacol 86(2–3):235–241

Pfaller MA, Diekema DJ (2007) Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev 20(1):133–163

Rahman MM, Alam AH, Sadik G, Islam MR, Khondkar P, Hossain MA, Rashid MA (2007) Antimicrobial and cytotoxic activities of *Achyranthes ferruginea*. Fitoterapia 78(3):260–262

Resende JCP, Resende MA (1999) *In vitro* antifungal susceptibility of clinical isolates of *Candida* spp. from hospitalized patients. Mycoses 42:641–644

Saleh MA, Belal MH, el-Baroty G (2006) Fungicidal activity of *Artemisia herba alba Asso* (Asteraceae). J Environ Sci Health B 41(3):237–244

Svetaz L, Agüero MB, Alvarez S, Luna L, Feresin G, Derita M, Tapia A, Zacchino S (2007) Antifungal activity of *Zuccagnia punctata* Cav.: evidence for the mechanism of action. Planta Med 73(10):1074–1080

Tan RX, Wolfender JL, Ma WG, Zhang LX, Hostettmann K (1996a) Secoiridoids and antifungal aromatic acids from *Gentiana algida*. Phytochemistry 41(1):111–116

Tan RX, Wolfender JL, Zhang LX, Ma WG, Fuzzati N, Marston A, Hostettmann K (1996b) Acyl secoiridoids and antifungal constituents from *Gentiana macrophylla*. Phytochemistry 42(5):1305–1313

Vazquez JA (2007) Combination antifungal therapy: the new frontier. Future Microbiol 2(2):115–139

Vollekova A, Kost'álová D, Kettmann V, Tóth J (2003) Antifungal activity of *Mahonia aquifolium* extract and its major protoberberine alkaloids. Phytother Res 17(7):834–837

Webster D, Taschereau P, René J, Sand C, Belland, Robert P, Rennie R (2008) Antifungal activity of medicinal plant extracts; preliminary screening studies. J Ethnopharmacol 115(1):140–146

Yenesew A, Derese S, Midiwo JO, Bii CC, Heydenreich M, Peter MG (2005) Antimicrobial flavonoids from the stem bark of *Erythrina burttii*. Fitoterapia 76(5):469–472

Zhai B, Lin X (2011) Recent progress on antifungal drug development. Curr Pharm Biotechnol 12(8):1255–1262

Chapter 6
Recent Advances on Medicinal Plants with Antifungal Activity

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Abstract Fungi are ubiquitous in the environment, and fungal infections have become more frequent. Medicinal plants are well-known natural sources for the treatment of various diseases since antiquity; in fact, despite emphasis being put in research of synthetic drugs, the body of literature identifying the antifungal potential of many traditional plants is growing mainly due to the fact that a lot of synthetic drugs are potentially toxic and are not free of side effects on the host. This chapter reviews the potential efficacy of natural products, either pure compounds, or plant extracts that provide unlimited opportunities to provide safe and efficient antifungal drugs.

Keywords Mycosis · Antifungal · Medicinal plants

6.1 Introduction

Human medicine has experienced a great development during the last decades; nonetheless, infections caused by microorganisms are still the cause of millions of human diseases. Those that are caused by fungi are called mycoses and show a wide variety of clinical signs; they usually represent painless subacute or chronic illness that begins after having contact with an environmental reservoir or from the patient's own flora. According to the affected area they can be classified as superficial, subcutaneous, or deep mycoses (Brandt and Warnock 2003).

When infection begins in healthy people it usually affects superficial areas such as the skin, hair, or nails; they are benign and are frequently solved spontaneously.

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They represent a major problem in those patients suffering from an impaired immunological system such as AIDS or immunosuppressive therapy because they may affect deeper tissues such as airways, kidneys, heart, or central nervous system (CNS) and even cause death (Ascioglu et al. 2002; Itin and Battegay 2012). The distribution of microorganism along the whole body may cause septicemia (Pemán and Salavert 2012).

Candida sp. represents the most frequent pathogenic fungi in human beings, mainly *C. albicans* (Calderone 2002; Sobel 2007). They cause diseases known as candidiasis that may affect oral cavity, sexual and deeper organs; they also may become chronic diseases in immunodeficient patients.

The oropharyngeal candidiasis itself is not clinically significant, but could be the focus of spread to other tissues or organs such as esophagus and interfere with normal nutrition or medicines intake.

Other interesting fungus because of being the cause of 25 % of fungal infections in humans is *Aspergillus* sp. (Calderone 2002; Sobel 2007; Karkowska-Kuleta et al. 2009; Chakrabarti and Singh 2011; Rotta et al. 2012); it usually affects superficial tissues such as impaired skin, nose, or ear ducts.

Dermatophytes such as *Microsporum*, *Epidermophyton,* and *Trichophyton* affect keratinized tissues of the organism (skin, nails, hair, or toes). These pathogens cause dermatophytoses (or tineas) that are not life threatening but are sometimes difficult to eradicate and may extend up to inflammatory lesions.

Deep mycoses with greater relevance are also systemic candidiases (*C. albicans*), aspergilloses (*A. fumigatus*, *A. flavus*, *A. niger*, *A. terreus*), or cryptococcoses (*Cryptococcus neoformans*) that may induce meningitis. Tropical areas are also affected by several other microorganisms that originate deep mycoses (Calderone 2002; Sobel 2007).

Therapeutic approach includes oral, intravenous, or topical medicines that modify the structure of the fungal cell wall by increasing its permeability (Walsh et al. 2008; Fortún 2011; Rotta et al. 2012). Amphotericin B, azolic drugs (fluconazole, ketoconazole), and enzymatic inhibitors such as caspofungin are some examples. The similar characteristics found between human cells and fungus derived from their eukaryotic nature makes it necessary to develop new molecules that are efficient in eliminating the pathogen but not affect human cells. The existence of polymorphic forms, such as yeast, plain cells, round cells, hyphae, pseudohyphae, spherulas, isolated cells, or mixed forms also creates difficulty in the development of an efficient drug.

Apart from the secondary effects and pharmacological interactions that may happen, chronic treatment may induce resistance (Pfaller 2012). As with several other diseases, developing countries are specially affected by mycoses due to the lack of specific effective medicines and the emergence of drug resistances.

All the above explained justify the need for having a wide range of molecules with proven antifungal efficacy and improved security.

Herbal remedies have been used since ancient times to treat fungal diseases, among others; many of them are still in the use in several traditional medicines, but most of them are lacking experimental assays that prove their antifungal

efficacy. So ethnopharmacological knowledge may represent a good source for safe and efficient antifungal drugs.

This chapter reviews the last advances in antifungal plants and their bioactive metabolites. A literature research was carried out including the last 10 years on the antifungal activity of plants and their active principles in the database Medline. The following keywords were applied: "antifungal; medicinal plant"; "antifungal; herbal"; "antifungal; medicine plant"; publication year 2002–2012; language English, French, or Spanish.

6.2 Recent Trends on Antifungal Properties of Medicinal Plants

6.2.1 Medicinal Plants with Antifungal Properties

Articles included in this chapter evaluate the *in vitro* or *in vivo* antifungal activity of herbal products in humans or experimental animals. Results are shown in Table 6.1; plants are listed under their botanical family that are alphabetically ordered; the used part, extract (or active principle), tested microorganism, and antifungal efficacy for each species are included, together with their bibliographic reference.

Some of the articles refer to the assay of antifungal activity of a high number of plants; only those species showing the strongest activity has been included in the table.

More than one thousand scientific publications have been found that fulfill any of the search criteria. Among them, there are 180 articles which have been selected because they completely fulfilled the scope of this chapter.

There are 91 botanical families representing 223 different species that have been recorded. Fabaceae is the one that contains the highest number of active species (23), followed by Asteraceae (15) and Lamiaceae. Finally, *Schinus terebinthifolius* Raddi (Anacardiaceae) and *Piper regnelli* (Miq) CDC (Piperaceae) are the species that have been the focus of the highest number of research works (but only three in each case) and demonstrated a potent antifungal activity.

No clinical trials with good quality have been found to prove antifungal efficacy of herbal products.

Most of the published studies refer to the efficacy toward pathogenic fungi in humans, mainly against *Candida* sp. A panel of standardized pathogenic yeasts and filamentous fungi such as *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, *Aspergillus niger,* and *Fusarium solani* is also included in the tests.

Table 6.1 Antifungal activity of selected medicinal plants

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Acanthaceae					
Justicia pectoralis var. *stenophylla* Leon.	LE	ME	*Candida krusei*	>500 mg/ml	Tempone et al. (2008)
Agapanthaceae					
Agapanthus africanus T.A. Duran and Hans Schinz	RH	EE Saponin	*Trychophyton mentagrophytes; Sporothrix schenekii*	15.6 μg/ml	Singh et al. (2008)
Agavaceae					
Sansevieria ehrenbergii Schweinf. Ex Baker	AP	Saponins	*Candida albicans* *Cryptococcus neoformans*	2–8 μg/ml 1–2 μg/ml	Pettit et al. (2005)
Aizoaceae					
Sesuvium portulacastrum L.	LE	Fatty acid methyl L esters extract	*Aspergillus fumigatus* *Aspergillus niger*	8 mg/ml	Chandrasekaran et al. (2011)
Alangiaceae					
Alangium salviifolium (L. f.) Wangerin subsp. *hexapetalum* (Wangerin)	Wood	Lyophilized extract	Dermatophytes, *C. albicans*	DIZ (mm) 25.23–14.78	Wuthi-udomlert et al. (2002)
Alliaceae					
Tulbaghia violacea Harv.	Bulbs	Petroleum ether DM	*C. albicans*	0.39 mg/ml 0.78 mg/ml	Ncube et al. (2011)
	LE	EE AAE		3.125 mg/ml 12.5 mg/ml	
Rhus tripartitum Ucria	AP	CE	*C. albicans*	0.07–0.62 mg/ml	Abbassi and Hani (2012)

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Anacardiaceae					
Anacardium occidentale L.	LE, FR	AA	*C. neoformans*	110 ng/ml	Schmourlo et al. (2005)
Harpephyllum cafrum Bernh.	SB	EE AA	*C. albicans*	0.78 mg/ml 1.56 mg/ml	Buwa and van Staden (2006)
Lithrea molleoides (Vell.) Engl.	AP	ME	*Microsporum canis* *M. gypseum* *Epidermophyton floccosum* *Trichophyton mentagrophytes* *T. rubrum*	250 μg/ml for every one	Muschietti et al. (2005)
Schinus molle L.	LE	AA	*C. albicans*	105 ng/ml	Schmourlo et al. (2005)
Schinus terebinthifolius Raddi	AP	AA	*C. albicans*	120 ng/ml	Schmourlo et al. (2005)
Schinus terebinthifolius Raddi	WP	ME	*C. albicans*	1.25 mg/ml	Braga et al. (2007)
Schinus terebinthifolius Raddi	LE SB	Schinol isolated from HE/DM	*Paracoccioides brasiliensis* strains Pb18, Pb3, Pb1578	7.5–12.5 μg/ml	Johann et al. (2010a)
Sclerocarya birrea (A.Rich.) Hochst.	RO	ME	*C. albicans* *C. parapsilosis* *C. tropicalis* *C. krusei* *C. neoformans*	250 μg/ml 125 μg/ml 63 μg/ml 63 μg/ml 250 μg/ml	Hamza et al. (2006)
Annonaceae					
Polyalthia longifolia cv *pendula*	SB, LE	Isolated 16-oxocleroda 3, 13E-dien-15-oic acid	*A. fumigatus* *Saccharomyces caulbequence* *S. cerevoceae* *C. albicans* *Hensila californica*	8–64 μg/ml	Katkar et al. (2010)

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Apiaceae					
Ferula assa-foetida	RO	Oleo-gum-resin	*A. parasiticus*	Inhibition of growth 24 %	Iranshahy and Iranshahi (2011)
Hydrocotyle bonariensis Lam.	LE	ME	*C. krusei*	137.79 mg/ml	Tempone et al. (2008)
Apocyanaceae					
Acokanthera schimperi (A.D.C.) Oliv	LE	ME	*T. mentagrophytes*	DIZ: 15–22 mm	Tadeg et al. (2005)
Plumeria bicolor	SB	CE	*Candida* sp *C. neoformans*	–	Singh et al. (2011)
Schizozygia coffaeoides (Boj.) Baill.	LE, RO bark	Alkaloids: 6,7-dehydro-19β-hydroxyschizozygine	*Trichophyton interdigitale* *T. mentagrophytes* *T. tonsurans* *Epidermophyton floccosum* *Microsporum gypseum* *Cladosporium cladosporioides* *C. herbarum*	1.95–15.6 µg/ml	Kariba et al. (2002)
Araceae					
Xanthosoma sagittifolium (L.) Schott	WP	AA	*Trichophyton rubrum*	100 ng/ml	Schmourlo et al. (2005)
Araliaceae					
Panax notoginseng (Burk) F.H. Chen	RO	Protein: Pananotin	*Coprinus comatus*, *Physalospora piricola* *Botrytis cinerea* *Fusarium oxysporum*	IC 50: 100 nM 1 µM 630 nM 560 nM	Lam and Ng (2002)

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Aristolochiaceae					
Aristolochia cymbifera Mart. and Zucc.	LE	ME	*C. krusei*	49.66 mg/ml	Tempone et al. (2008)
Asclepiadaceae					
Cynanchum komarovii Al Iljinski	SE	Antifungal protein thaumatin-like (TLP)	*Verticillium dahlia*	IC_{50} (μM) 24	Wang et al. (2011)
			F. oxysporum	20	
			Rhizoctonia solani	21	
			B. cinerea	11	
			Valsa malo	<3.0	
Vincetoxicum stocksii Ali and Khatoon	Whole plant	ME	*C. albicans*	MIC 85 % (μg/ml): 200	Zaidi et al. 2005 1.56 mg/ml
Asteraceae					
Baccharis dracuncufolia DC	–	HE fractions of HA	*Paracoccioides brasiliensis*	7.8 μg/ml	Johann et al. (2010b)
Baccharis grisebachii	Resinous exudate	Exudate (**1**)	*Microsporum canis* *Microsporum gypseum*	(**1**) 50, 100, 50, 50, and 50 μg/ml, respectively	Feresin et al. (2003b)
		labda-7,13*E*-dien-2*β*,15-diol (diterpene) (**2**)	*Epidermophyton floccosum*	(**2**) 25, 50, 12.5, 12.5, and 25 μg/ml, respectively	
		p-coumaric acid derivatives: 3-prenyl-*p*-coumaric acid (**3**), 3,5-diprenyl-*p*-coumaric acid (**4**)	*T. rubrum* *T. mentagrophytes*	(**3**) 100, 100, 50, 100, and 100 μg/ml, respectively (**4**) 100, 125, and 125 μg/ml, towards *E.f.*, *T.r.* and *T.m.*, respectively	

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Baccharis trimera (Less.) DC.	LE	ME	*C. krusei*	187.50 mg/ml	Tempone et al. (2008)
Chuquiraga spinosa (R y P) Don	AP	ME:H_2O (50:50)	*C. albicans*	6.25 μg/ml	Casado et al. (2011)
		AAE	*C. albicans*	2.5 μg/ml	
			Cladosporium cucumerinum	2.5 μg/ml	
Chromolaena odorata (L.) R.M. King and H.Rob.	LE	AA/EE	*C. neoformans Microsporum gypseum*	62.5–600 μg/ml	Ngono et al. (2006)
			T. mentagrophytes		
			T. rubrum		
Echinops ellenbeckii O.Hoffm.	FL	AA/EE	*C. albicans*	Strong	Hymete et al. (2005)
Eupatorium aschenbornianum Schauer	AP	Benzofurane compounds: 5-acetyl-3beta-angeloyloxy-2beta-(1-hydroxyisopropyl)-2,3-dihydrobenzofurane (**1**); 5-acetyl-3beta-angeloyloxy-2beta-(1-hydroxyisopropyl)-6-methoxy-2,3-dihydrobenzofurane (**2**); espeletone (**3**), encecalinol (**4**)	*T. mentagrophytes*	(**1**) 200 μg/ml	Ríos et al. (2003)
			T. rubrum	(**2**) 50 μg/ml	
			C. albicans	(**3**) 100 μg/ml	
			A. niger	(**4**) 12.5 μg/ml	
				(**1**) 100 μg/ml	
				(**2**) 50 μg/ml	
				(**3**) 100 μg/ml	
				(**4**) 12.5 μg/ml	
				(**4**) 100 μg/ml	
				(**4**) 200 μg/ml	
Eupatorium aschenbornianum Schauer	L Steam	(1)HE/(2)ME	*C. albicans*	(1) 8.0/(2) 8.0 μg/ml	Navarro García et al. (2003)
			A. niger	(1) 4.0/(2) 8.0 μg/ml	
			T. mentagrophytes	(1) 0.03/(2) 1.0 μg/ml	
			T. rubrum	(1) 0.2/(2) 0.5 μg/ml	

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Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Gnaphalium gaudichaudianum DC			*Sporothrix schenckii* *Fonseca pedrosoi*	50 µg/ml 12.5 µg/ml	Gaitán et al. (2011)
Gymnosperma glutinosum (Spreng.) Less.		HE	*T. rubrum*	IC(50) = µg/ml 23.79 90.25	Canales et al. (2007)
Ophyrosporus peruvianus (Gmelin) King and H. Rob.	L Steam	95 % EE	*C. albicans* *T. mentagrophytes* *M. gypseum* *Sporothrix schenckii*	DIZ (mm) (0–27)	Rojas et al. (2003)
Pterocaulon alopecuroides (Lam.)DC	AP	ME	*C. albicans*	25 to >800 µg/ml	Stein et al. (2005)
P. interruptum DC.					
P. polystachyum DC.					
Tagetes lucida CAV.	AP	ME	*A. niger* Fusarium moniliforme Fusarium sporotrichum Rhizoctonia solani	DIZ (mm) 15.5–25.3 23, 1–40, 4 17, 9–35, 1 8, 1–16, 4	Céspedes et al. (2006)
Vernonia cinerea Less.	LE	ME	*C. albicans*	1.56 mg/ml	Latha et al. (2011)
Wedelia paludosa DC. (*Acmela brasiliensis*)		HE/DE/BE fractions	Dermatophytes: E. floccosum T. rubrum T. mentagrophytes	250–1,000 µg/ml	Sartori et al. (2003)
Balanophoraceae					
Thonningia sanguinea Vahl.	WP	EA	*C. neoformans*	IC_{50}: 0.06 mg/ml	Ouattara et al. (2007)

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Berberidaceae					
Berberis aristata Roxb. Ex DC	RO	EE	*A. fumigatus; Fusarium moniliforme; F. semitectum; Pytium* sp.	10; 12; 15; 15 μg/ml	Sharma et al. (2008)
		CE	*Blastoschizomyces capitatus*	22 μg/ml	
Mahonia aquifolium (Pursh) Nutt.	SB	Crude extract berberine, palmatine jatrorrhizine	Dermatophytes	500 to ≥1,000 μg/ml	Volleková et al. (2003)
			Candida sp.	500 to ≥1,000 μg/ml	
				500 to ≥1,000 μg/ml	
				62.5–125 μg/ml	
Bignoniaceae					
Markhamia tomentosa (Benth.)K.Schum.	LE	ME	*Candida pseudotropicalis*	DIZ(mm): 3 225 mg/ml	Aladesanmi et al. (2006)
Boraginaceae					
Arnebia benthamii Wall. ex G.Don	RO	EE	*A. fumigatus; Blastoschizomyces capitatus; Fusarium semitectum; Rhizopus* sp.	10; 22; 22; 22 μg/ml	Sharma et al. (2008)
		EAE	*A. fumigatus*	10 μg/ml	
		CE	*A. versicolor; Blastoschizomyces capitatus*	22; 22 μg/ml	
		HE	*A. versicolor; B. capitatus; F. semitectum; Rhizopus* sp.	22; 22; 22; 15 μg/ml	
Bourreria huanita (Lex.) Hemsl	–	–	*Sporothrix schenckii*	12.5 μg/ml	Gaitán et al. (2011)
			Fonseca pedrosoi	25 μg/ml	

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Lithospermum erythrorhizon Siebold and Zuccarini		Naphtoquinone derivatives: Deoxyshikonin (**1**), Acetylshikonin (**2**), *β*-hydroxyisovaleryl shikonin (**3**), shikonin (**4**)	*C. albicans, C. albicans* (azole resistant), *C. glabrata, C. krusei, C. tropicalis, C. parapsilosis, Saccharomyces cerevisiae, C. neoformans, T. cutaneum, A. fumigatus*	(**1**) 2 to >64 μg/ml (**2**) (8–32) μg/ml (**3**) (16–64) μg/ml (**4**) (8 to >64) μg/ml	Sasaki et al. (2002)
Brassicaceae					
Brassica campestris L.	SE	Peptide	*Fusarium oxysporum; Mycosphaerella arachidicola*	IC_{50}: 8.3 μM;4.5 μM	Lin et al. (2007)
Brassica oleracea L. var. *capitata* f. *rubra* DC	LE	ME	*T. rubrum*	300 mg/ml	Hafidh et al. (2011)
			A. terreus	150 mg/ml	
Isatis tinctoria L.	WP	AF	*T. schoen leinii; Trichophyton simii; A. niger; C. albicans; Macrophomina phaseolina*		Ahmad and Fatima (2008)
Buxaceae					
Buxus hildebrandtii Baill.	LE	ME	*Candida maltosa*	DIZ(mm): 25	Mothana and Lindequist (2005)
Caesalpinaceae					
Cassia fistula L.	SE Fruit pulp	EE	*C. albicans*	350 μg/ml	Irshad et al. (2011)
			C. tropicalis	300 μg/ml	
			C. glabrata	300 μg/ml	
			C. albicans	150 μg/ml	
			C. tropicalis	250 μg/ml	
			C. glabrata	100 μg/ml	

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Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Caryophyllaceae					
Psammosilene tunicoides W.C. Wu and C.Y. Wu	RO	Isolated protein	*C. albicans SC5314* *C. albicans Y0109* *C. tropicalis* *C. parapsilosiS* *C. neoformans BLS108*	4.0 μg/ml 16.0 μg/ml 0.25 μg/ml 1.0 μg/ml 1.0 μg/ml	Tian et al. (2010)
Celastraceae					
Elaedodendron buchannanii (Loes.)Loes.	SB	ME	*C. tropicalis* *C. krusei* *C. neoformans*	250 μg/ml 63 μg/ml 31 μg/ml	Hamza et al. (2006)
Clusiaceae					
Calophyllum brasiliensis L. Cambess.	RO	DM	*C. albicans* *C. krusei*	7.81 μg/ml	Albernaz et al. (2010)
Combretaceae					
Anogeissus latifolia (Roxb. Ex DC.) Wall. Ex Guill. and Perri.	SB	AA/EE	*C. Albicans* *C. albidus* *A. flavus* *T. rubrum*	3.29 μg/ml 6.81 μg/ml 31.25 μg/ml 125 μg/ml	Govindajaran et al. (2006)
Anogeissus leiocarpus (DC) Guill. and Perr.	RO	ME EE CE AE EAE	*A. niger, A. fumigatus, Penicillium species, Microsporum audouinii T. rubrum*	0.03–0.07 μ/ml	Mann et al. (2008)
Anogeissus leiocarpus (DC) Guill.and Perr.	LE, SB, RO	EE	10 Yeasts 10 Filamentous micromycetes	0.25–4 mg/ml	Batawila et al. (2005)

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Combretum sp.	LE, RO, SB	ME (50 mg/ml)	*C. albicans*	DIZ (mm): (16–26)	Fyhrquist et al. (2002)
Combretum caffrum Kuntze	Bark	AE (5 and 10 mg/ml)	*Alternaria alternaria*	Growth inhibition (%): 62.0 and 100.0	Masika and Afolayan (2002)
			A. niger	29.0 and 58.0	
			Mucor hiemalis	92.9 and 100.0	
			Penicillium notatum	36.3 and 49.3	
			Schizophyllum commune	73.3 and 100.0	
		ME (5 and 10 mg/ml)	*A. alternaria*	62.3 and 100.0	
			A. niger	61.3 and 100.0	
			Mucor hiemalis	86.7 and 100.0	
			Penicillium notatum	65.7 and 100.0	
			Schizophyllum commune	100.0 and 100.0	
		AAE (5 and 10 mg/ml)	*A. alternaria*	16.0 and 19.7	
			A. niger	−44.0 and −39.0	
			Mucor hiemalis	76.3 and 84.0	
			Penicillium notatum	8.7 and 12.0	
			Schizophyllum commune	25.7 and 34.3	
		Decoction extracts (5 and 10 mg/ml)	*A. alternaria*	46.1 and 57.1	
			A. niger	40.6 and 48.4	
			Mucor hiemalis	95.6 and 94.6	
			Penicillium notatum	50.0 and 57.9	
			Schizophyllum commune	62.3 and 62.3	
Guiera senegalensis J. F. Gmel	LE	Guieranone A (methoxylated naphthyl butanone)	*Cladosporium cucumerinum*	Minimum amount for inhibition of fungal growth on TLC (μg): 1	Silva and Gomes (2003)

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Terminalia spp. (*T. prunioides, T. brachystemma, T.sericea, T.gazensis, T. mollis, T. sambesiaca)*	LE,RO,SB	ME, EE, AE, AAE (50 mg/ml)	*C. albicans*	DIZ(mm): 24–33	Fyhrquist et al. (2002)
		AE	*C. albicans* *C. neoformans* *A. fumigatus Microsporum canis* *Sporothrix schenkii*	MIC (mg/ml): (0.02–0.08)	Masoko et al. (2005)
Terminalia avicennioides Guill and Perr	RO	ME EE CE AE EAE	*A. niger, A. fumigatus, Penicillium* species, *Microsporum audouinii* *T. rubrum*	0.03–0.07 μ/ml	Mann et al. (2008)
Terminalia chebula (Gaertner) Retz.	SE	ME	*C. albicans* Clotrimazole-resistant	0.62 mg/ml	Bonjar (2004)
Terminalia glaucescens Planchon.	LE, RO	EE	10 Yeasts 10 Filamentous micromycetes	0.25–4 mg/ml	Batawila et al. (2005)
Terminalia laxiflora Engl.	LE	EE	10 Yeasts 10 Filamentous micromycetes	0.25–4 mg/ml	Batawila et al. (2005)
Terminalia macroptera Guill. and Perr.	LE, SB, RO	EE	10 Yeasts 10 Filamentous micromycetes	0.25–4 mg/ml	Batawila et al. (2005)
Terminalia sericea Burch. Ex. DC	RO	AA/EE	 *C. albicans* *A. niger*	DIZ (mm): 15 22	Moshi and Mbwambo (2005)
Terminalia triflora (Griseb) Lillo	AP	ME	*M. gypseum* *T. mentagrophytes* *T. rubrum*	250 μg/ml 100 μg/ml 100 μg/ml	Muschietti et al. (2005)

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Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Commelinaceae					
Commelina diffusa Burn.	WP	ME	*Trichophyton* spp.		Mensah et al. (2006)
Cornaceae					
Curtisia dentata Aiton	LE SB	Pentacyclic triterpenoids	*C. neoformans, Sporothrix schenckii, A. fumigatus, Microsporum canis and C. albicans*	8–63 μg/ml	Shai et al. (2008)
Costaceae					
Costus speciosus	RH	Isolated Costunolide	*T. mentagrophytes* *T. simii* *T. rubrum* 296 *T, rubrum* 57 *E. flocosum* *Scopulariopsis sp.* *A. niger* *Cunvulari lunata* *Magnaporthe grisea*	62.5 μg/ml 62.0 μg/ml 31.25 μg/ml 62.5 μg/ml 125 μg/ml 250 μg/ml 250 μg/ml 125 μg/ml 250 μg/ml	Duraipandiyan et al. (2012)
Crassulaceae					
Sedum oxypetalum HBK.	L Steam	HE/ME	C. albicans A. niger T. mentagrophytes T. rubrum	8.0/>8.0 μg/ml 8.0/8.0 μg/ml 8.0/8.0 μg/ml 8.0/2.0 μg/ml	Navarro García et al. (2003)

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Cucurbitaceae					
Coccinia grandis (L.) Voigt	LE	AAE	*C. albicans*	1,000 μg/ml	Bhattacharaya et al. (2010)
		EE	*C. albicans*	750 μg/ml	
			A. niger	1,000 μg/ml	
Momordica charantia L.	LE, F	AA	*C. albicans*	150 ng/ml	Schmourlo et al. (2005)
			C..s neoformans	180 ng/ml	
Momordica charantia L.	LE	EE	*C. albicans*	≤1,024 μg/ml	Santos et al. (2012)
			C. tropicalis	IC_{50} 46.06 μg/ml	
			C. krusei		
Cupressaceae					
Cupressus lusitanica Mill.	LE	HE	*Microsporum audouinii*	750 μg/ml	Kuiate et al. (2006)
			M. langeronii	750 μg/ml	
			M. canis	750 μg/ml	
			T. rubrum	800 μg/ml	
Dilleniaceae					
Curatella americana L.	SB	Brazilian cachaca extract	C. albicans	31.3 μg/ml	De Toledo et al. (2011)
			C. parapsilosis	31.3 μg/ml	
Dipterocarpaceae					
Hopea exalata Lin, Yang and Hsue	SB	Stilbenoids	*Alternaria attenata*	0.78–22.2 μg/ml	Ge et al. (2006)
			A. solani		
			Colletotrichum lagenarium		
			F. oxysporum		
			Pyriculariaoryzae		
			V. mali		

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Ebenaceae					
Diospyros crassifolia H. Perrier	SB	ME DME Plumbagin	*C. albicans, C. glabrata, C. krusei, C. tropicalis, C. neoformans, A. niger, A. flavus, Alternaria* sp., *Cladosporium* sp., *Geotrichum candidum, Fusarium* sp.; *Penicillium* sp.	12.5–25 mg/ml (extracts) 0.78–3.12 μg/ml (plumbagin)	Dzoyem et al. (2007)
Elaeocarpaceae					
Eleacarpus panitrus Roxb.	Ripe fruit	CE EE	*C. albicans* *A. niger*	1.5 mg/ml 3.0 mg/ml	Singh et al. (2010)
Euphorbiaceae					
Euphorbia prostrata	AP	HE AE BE	*C. albicans*	63 μg/ml 16 μg/ml 31 μg/ml	Alanís-Garza et al. (2007)
Hevea brasiliensis (Willd. Ex A. Juss) Müll. Arg.	Latex	Latex—C serum	*A. niger*	2.5 mg/ml	Daruliza et al. (2011)
Mallotus oppositifolius (Geisel.) Müll.		AA	*Penicillium* sp.	DIZ (mm): 24.75	Adekunle and Ikumapayi (2006)
Phyllanthus piscatorum Kunth.	AP	AA;ME;DE	*A. fumigatus* *A. flavus* *C. albicans*	DIZ (mm): >1	Gertsch et al. (2004)
Sebastiania brasilensis Spreng. *S. commersoniana* (Baill.) L.B.Sm.and Downs	AP	ME	*M. canis* *M. gypseum* *E. floccosum* *T. mentagrophytes* *T. rubrum*	250 μg/ml 1000 μg/ml 250 μg/ml 500 μg/ml 500 μg/ml	Muschietti et al. (2005)

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Fabaceae					
Acacia confusa Merr.	SE	Isoalted Acaconin	*Rhizoctonia solani*	IC_{50} :30 ± 4 μM	Lam and Ng (2010a)
Acacia confusa Merr.	SE	Isoalted Acafusin	*R. solani*	IC_{50} :28 μM	Lam and Ng (2010b)
Acacia robusta Burch.	LE	ME	*Candida parapsilosis* *C. krusei*	63 μg/ml 31 μg/ml	Hamza et al. (2006)
Acacia nilotica (L.) Del.	SB	ME	*C. parapsilosis* *C. tropicalis*	31 μg/ml 63 μg/ml	Hamza et al. (2006)
Albizia inundata (Mart.) Barneby and J.W. Grimes	LE	ME	*C. krusei*	49.66 mg/ml	Tempone et al. (2008)
Albizia myriophylla Benth.	ST	ME	*Candida* sp.	100–500 mg/ml	Rukayadi et al. (2008)
Bauhinia forficata Link.	LE	ME	*C. krusei*	>500 mg/ml	Tempone et al. (2008)
Bauhinia purpurea L.	RO	dihydrodibenzoxepins; dihydrobenzofuran; Spirochromane-2, 1′-hexenedione; bibenzyl	*C. albicans*	IC_{50} 49.6–130.1 μM	Boonphong et al. (2007)
Caesalpinea pyramidalis Tul.	WP	AAE	*T. rubrum; C. guilliermondii; C. albicans; C. neoformans; F. pedrosoi*	6.5 μg/ml	Cruz et al. (2007)
Cajanus cajan L.(Millsp)	WP	EM	*C. albicans*	1.25 mg/ml	Braga et al. (2007)
Calia secundiflora (Ortega) Yakovlev	SE	OE	*Alternaria solani; F. oxysporum, Monilia fructicola*		Pérez-Láinez et al. (2008)

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Cassia fistula L.	FL	AAE ME EAE CE HE 4-Hydroxy benzoic acid	*T. mentagrophytes; E. floccosum*	0.5; 0.5 mg/ml	Duraipandiyan and Ignacimuthu (2007)
Cassia spectabilis DC.	LE	ME	*C. albicans*	6.25 mg/ml	Torey and Sasidharan (2011)
Cicer arietinum L.	SE	Cicerarin (peptide) 40 μg		IC_{50} (μM)	Chu et al. (2003)
			Botrytis cinerea	20.6	
			Mycosphaerella arachidicola	15.3	
			Physalospora piricola	8.2	
Daniellia oliveri (Rolfe) Hutch. and Dalz.	Leaves	BE		DIZ (mm):	Ahmadu et al. (2004)
			T. rubrum	10.5	
Entada abysinnica Steudel ex A. Rich	LE	ME	*C. albicans*	1.875 mg/50 μl	Mariita et al. (2010)
Gleditsia sinensis Lam.		Ellagic acid glycosides	*Magnaporthe grisea*	IC_{50} 13.56 μg/ml	Zhou et al. (2007)

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Lupinus angustifolius L.	SE	Three saponins: 3*β*,21*β*,22*β*,24-tetrahydroxyolean-12-en-3-*O*-α-l-rhamnopyranosyl-[α-l-rhamnopyranosyl]-*β*-d-galactopyranosyl-*β*-d-glucuronopyranoside; 3*β*,21*β*,22*β*,24-tetrahydroxyolean-12-en-3-*O*-α-l-rhamnopyranosyl-[α-l-rhamnopyranosyl]-*β*-d-galactopyranosyl-*β*-d-glucuronopyranosyl-21-*O*-α-l-rhamnopyranoside; 3*β*,21*β*,22*β*,24-tetrahydroxyolean-12-en-3-*O*-α-l-rhamnopyranosyl-*β*-d-galactopyranosyl-*β*-d-glucuronopyranosyl-21-*O*-α-l-rhamnopyranoside	*C. albicans*	2, 3 and 4 showed moderate antifungal with MIC 25, 30 and 25 μg/ml, respectively	Woldemichael and Wink (2002)
Lysiloma acapulcensis (Kunth) Benth.	SB	ME	*C. albicans* *A. niger* *T. mentagrophytes* *T. rubrum*	2.0 μg/ml 4.0 μg/ml 1.0 μg/ml 1.0 μg/ml	Navarro García et al. (2003)
Machaerium multiflorum Spruce	SB	5,6-*seco*-hexahydrodibenzopyrans: Machaeridiol A (**1**) Machaeridiol B (**2**)	*C. albicans*	IC_{50}/MIC (μg/ml) (**1**) 3.5/50 (**2**) 2.0/6.25	Muhammad I et al. (2003)
Peltophorum pterocarpum (DC.) K. Heyne		ME	*C. albicans*	DIZ (mm): 9–15	Duraipandiyan et al. (2006)

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Phaseolus vulgaris L.	SE	Isolated 64-kDa hemagglutinin	*V. mali*	IC_{50} 10 μM	Lam and Ng (2010c)
Plathymeria reticulate Bth.	SB	Brazilian cachaca extract	*C. albicans*	62.5 μg/ml	De Toledo et al. (2011)
			C. parapsilosis	62.5 μg/ml	
Schotia latifolia Jacq.	bark			Growth inhibition (%)	Masika and Afolayan (2002)
		AE Conc. 5 and 10 mg/ml	*A. alternaria*	51.0 and 69.3	
			A. niger	5.7 and 12.7	
			Mucor hiemalis	83.3 and 100.0	
			Penicillium notatum	7.7 and 10.3	
			Schizophyllum commune	24. and 61.7	
		ME Conc. 5 and 10 mg/ml	*A. alternaria*	51.0 and 69.3	
			A. niger	5.7 and 12.7	
			M. hiemalis	83.3 and 100.0	
			P. notatum	7.7 and 10.3	
			S. commune	24. and 61.7	
			A. alternaria	59.0 and 100.0	
			A. niger	64.3 and 100.0	
			M. hiemalis	73.7 and 100.0	
			P. notatum	49.0 and 100.0	
			S. commune	63.0 and 88.3	
		AAE Conc. 5 and 10 mg/ml	*A. alternaria*	19.3 and 23.0	
			A. niger	6.3 and 4.3	
			M. hiemalis	49.3 and 57.3	
			P. notatum	4.0 and 4.3	
			S. commune	14.0 and 1.7	

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Fagaceae					
Castanea mollisima Blume	SE	Protein: mollisin	*F. oxysporum* *Mycosphaerella arachidicola* *Physalospora piricola*	IC_{50} (μM) 0.83 6.48 9.21	Chu and Ng (2003)
Gymnocladus chinensis Baillon	SE	Peptide	*F. oxysporum* *M. arachidicola*	IC(50) (μM): 2 10	Wong and Ng (2003)
Flacourtiaceae					
Casearia sylvestris Sw.	LE twigs	Clerodane diterpenoids: casearvestrin A casearvestrin B casearvestrin C	*A. niger*	DIZ(mm^2) A: 348 ± 38 B: 296 ± 36 C: 177 ± 49 EC_{50} (μg/ml) A: 1.4 ± 0.7 B: 1.4 ± 0.5 C: 0.34 ± 0.03,	Oberlies et al. (2002)
Gracilariaceae					
Gracilaria changii B.M. Xia and I.A. Abbott	WP	ME	*C. albicans*	1.56 mg/ml	Sasidharan et al. (2011)
Herreriaceae					
Herreria salsaparilha Mart.	LE	ME	*C. krusei*	>500 mg/ml	Tempone et al. (2008)
Hidrofilaceae					
Wigandia urens (R. and P.) Kunth	L Steam	95 % ethanol extract	*C. albicans* *T. mentagrophytes* *M. gypseum* *Sporothrix schenckii*	DIZ (mm) 13–19 0–13	Rojas et al. (2003)

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Hypericaeae					
Hypericum perforatum L. (St. John's Wort)	AP	10 % decoct	*C. albicans*	DIZ (mm): 8	Kovac-Besovic et al. (2003)
Iridaceae					
Eleutherine bulbosa (Mill.) Urb.	LE	ME	*C. krusei*	>500 mg/ml	Tempone et al. (2008)
Juglandaceae					
Juglans regia L.	SB	EE	*Blastoschizomyces capitatus*	22 mg/ml	Sharma et al. (2008)
Lamiaceae					
Calamintha adscendens Willk. and Lang.	LE	ME	*C. krusei*	IC_{50} 112.20 µg/ml	Tempone et al. (2008)
Mentha X piperita L.	LE	ME	*C. krusei*	>500 µg/ml	Tempone et al. (2008)
Micromeria cilicica Hausskn. ex P.H.Davis	AP	Extracts–Essential oil	*C. albicans*	DIZ (mm): (13–34)	Duru et al. (2004)
Ocimum gratissimum L.	WP	ME	*C. albicans* *C neofromans*	1.25 mg/ml 0.078 mg/ml	Braga et al. (2007)
Ocimum forskolei Benth.	WP	ME	*T.mentagrophytes*	Inhibition zone diameter (mm): 30	Al-Fatimi et al. (2007)
Plectranthus amboinicus (Lour.) Spreng.	LE	ME	*C. krusei*	>500 µg/ml	Tempone et al. (2008)
Plectranthus barbatus Andrews	LE	ME	*C. krusei*	49.64 µg/ml	Tempone et al. (2008)

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Plectranthus grandis (Cramer) R.H. Willemse	LE	ME	*C. krusei*	>500 μg/ml	Tempone et al. (2008)
Plectranthus neochilus Schlechter	LE	ME	*C. krusei*	>500 μg/ml	Tempone et al. (2008)
Salvia texana Scheele Torr.	AP	AE	*C. albicans; A. fumigatus; Coccidioides immitis; Hisoplasma capsulatum*	62; 125; 16; 16 μg/ml	Alanís-Garza et al. (2007)
		EB		125; 250; 63; 32 μg/ml	
Satureja khuzistanica Jamzad	AP	ME	*A. niger*	2–8 μg/ml	Amanlou et al. (2004)
			C. albicans	1–4 μg/ml	
Thymus pulegioides L.	AP	ME	*F. oxysporum*	DIZ (mm): 11MIC (μg/ml):	Pinto et al. (2006)
		Essential oil	*Candida* spp	0.32–0.64	
			Aspergillus spp	0.16–0.32	
			Dermatophytes:		
			M. canis	0.16	
			M. gypseum	0.16	
			T. rubrum	0.32	
			T. mentagrophytes	0.16	
			E. floccosum	0.16	
Thymus vulgaris L.	WP	MeOH(20 mg/ml)	*C. albicans*	0.62 μg/ml	Bonjar (2004)
Vitex negundo L.	LE	Flavone glycoside	*T. mentagrophytes*	6.25 μg/ml	Sathiamoorthy et al. (2007)
			C. neoformans		
Lauraceae					
Aniba panurensis (Meisn.) Mez	Wood	Alkaloid	*Candida* azole resistant	0.25–4 μg/ml	Klausmeyer et al. (2004)
			C. neoformans		

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Ocotea odorífera (Vellozo) Rohwer	LE	Isolated ellagitanin	*C. parapsolosis*	1.6 μg/ml	Yamaguchi et al. (2011)
Leguminosae					
Caesalpinia bonducella F.	AP	n-Butanol	*C. glabrata*	Inhibition of growth 80 %	Khan et al. (2011)
		CE	*A. flavus*	Inhibition of growth 70 %	
		CRE	*C. glabrata*	Inhibition of growth 70 %	
			A. flavus	Inhibition of growth 65 %	
Ononis spinosa L.	RO	AAE	*C. albicans*	1.25 μg/ml	Altuner et al. (2010)
	AP	EE	*C. glabrata*	5.00 μg/ml	
Liliaceae					
Allium ursinum L.	LE	Isolated allicin	*C. albicans*	0.5 mg/ml	Bagiu et al. (2012)
			C. famata	1.15 mg/ml	
			C. glabrata	1.33 mg/ml	
			C. krusei	1 mg/ml	
Smilax medica Schltdl et Cham.	RO	Saponins	C. albicans C. glabrata C. tropicalis	MIC (μg/ml): (12.5–50)	Sautour et al. (2005)
Lithraceae					
Punica granatum L.		ME	*C. albicans*	DIZ (mm): 9–13	Duraipandiyan et al. (2006)
Malvaceae					
Malva parviflora L.	RO	ME	*T. mentagrophytes*	DIZ (mm): 16–28	Tadeg et al. (2005)

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Melanthiaceae					
Paris polyphylla Smith.	RH	Steroidal saponins	*Cladosporium cladosporioides; Candida* spp.	–	Deng et al. (2008)
Meliaceae					
Amoora rohituka Roxb.	SB	**PE:** [360 μg/ml] **DM**: [360 μg/ml] **ME** [360 μg/ml]	*Macrophomina phaseolina*	% Inhibition of fungal mycelial growth	Chowdhury et al. (2003)
				PE: 19.73	
				DM: 11.67	
				ME: 7.85	
			Botryodiplodia theobromate	PE: 35.94	
				DM: 32.11	
				ME: 15.85	
			Curvularia lunata	PE: 34.21	
				DM: 35.46	
				ME: 17.76	
Toona ciliate M. Roem.	SB	Petrol ether [570 μg/ml] (**PE**), dichoromethane [360 μg/ml] (**DM**), methanol extracts [360 μg/ml] (**ME**)	*M. phaseolina*	% Inhibition of fungal mycelial growth	Chowdhury et al. (2003)
				DM: 10.28	
				ME: 8.33	
			B. theobromate	DM: 30.49	
				ME: 30.49	
			C. lunata	PE: 32.24	
				DM: 16.12	
				ME: 12.28	
Trichila heudelotii Planch. Ex Oliv.	LE	ME	*C. albicans*	DIZ (mm): 3	Aladesanmi et al. (2006)
			C. pseudotropicalis	1	
			T. rubrum	2	

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Turraea holstii Gürke.	LE	ME		MIC (μg/ml):	Hamza et al. (2006)
			C. tropicalis	63	
			C. krusei	500	
			C. neoformans	125	
Melyanteaceae					
Bersama lucens (Hochst.) Szyszyl.	SB	EE	C. albicans	MIC (mg/ml): 0.78	Buwa and van Staden (2006)
Menispermaceae					
Albertisia villosa (Exell) Forman	ROB	Alkaloid: cyanine		Inhibition %	Lohombo-Ekomba et al. (2004)
			Trichophyton longiformis	100	
			C. albicans	81.25	
			A. flavus	100	
			Microsperum canis,	93.9	
			Fusanum solani var *lycopersici-tomato Moniliform* spp.	91.86 100	
Anomospermum grandifolium Eichler ("Icu")	ST	CE; jujubogenin glycosides	*C. albicans*	200/250 μg/ml	Plaza et al. (2003)
Sphenocentrum jollyanum (SJ) Pierre.	RO	ME	*C. albicans*	DIZ (mm): 225 mg/ml 5	Aladesanmi et al. (2006)
			C. pseudotropicalis	6	
			T. rubrum	3	

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Moraceae					
Artocarpus heterophyllus Lam.	Latex	Latex	*C. albicans*	2.2 mg/ml	Siritapetawee et al. (2012)
Broussonetia papyrifera (L.) Vent.	LE	Isolated proteins PMAPI, PMAPII	*Trichoderma viridi*	IC_{50} 0.1 μg/ml	Zhao et al. (2011)
Cudrania cochinchinensis Lour.	RO	Cudraxanthone S (**1**), Toxyloxanthone C (**2**) wighteone (**3**)	*A. fumigatus, A. nidulans, C. neoformans* *Candida glabrata*	(**1**) (**2**) (**3**): 2–8 μg/ml (**1**) (**3**): 4–8 μg/ml	Fukai et al. (2003)
Ficus carica L.	Latex	Latex	*C. albicans*	Inhibition of growth at 500 μg/ml	Aref et al. (2010)
Myrtaceae					
Eugenia jambolana Lam.	FR	EE	*C. albicans* *C. tropicalis* *C. krusei*	≤1,024 μg/ml	Dos Santos et al. (2012)
Eugenia uniflora L.	LE	hydroalcoholic extract lyophilized	*C. krusei* *C. parapsilosis* *C. tropicalis*	31.2 μg/ml 125 μg/ml 31.2 μg/ml	Holetz et al. (2002)
Melaleuca leucadendron L	BR	EtOH	*M. canis*	IC_{50} 13 mg/ml	Valdés et al. (2008)
Myrtus communis L.	LE SE	ME	*C. albicans* Clotrimazole-resistant	10 mg/ml	Bonjar (2004)

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Psidium guajava L.	LE	hydroalcoholic extract lyophilised	*C. albicans* *C. krusei* *C. parapsilosis* *C. tropicalis*	125 μg/ml 15.6 μg/ml 62.5 μg/ml 15.6 μg/ml	Holetz et al. (2002)
Syzygium cumini (L.) Skills	WP	ME	*C. neoformans*	0.078 mg/ml	Braga et al. (2007)
Olacaceae					
Ximenia americana L.	SB,LE	ME(**1**);AAE (**2**)	*C. albicans Saccharomyces cerevisiae A. niger*	(**1**) 1.23–19.79 μg/ml (**2**) 0.29–49.84 μg/ml	Omer and Elnima (2003)
Oliniaceae					
Olinia rochetiana A.Juss.	LE	ME	 *C. albicans* *T. mentagrophytes*	DIZ (mm): 14–18 18–54	Tadeg et al. (2005)
Onagraceae					
Epilobium angustifolium L.	RO	AE	*Candida sp.; C. neoformans*	IC_{80} 25–1,600; 400 mg/ml	Webster et al. (2008)
Oenothera multicaulis R. and P.	RO Aerial part	95 % EE	*C. albicans* *T. mentagrophytes* *M. gypseum* *Sporothrix schenckii*	IZD (mm) (0–19) (0–15)	Rojas et al. (2003)
Ophioglossaceae					
Ophioglossum pedunculosum L.	RO	Purified lectin	*Scletorium rolfsii* *Fusarium gramineum*	Inhibition of growth at 40–70 μg/ml	He et al. (2011)

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Oxalidaceae					
Oxalis erythrorhiza Gillies ex Hooker	Aerial parts	Benzoquinone: Embelin	*E. floccosum* *M. canis* *M. gypseum* *T. mantagrophytes* *Trichiphytum rubrum*	50–100 μg/ml	Feresin et al. (2003a)
Myristicaceae					
Iryanthera lancifolia Ducke Suesseng.	Steam	95 % EE	*C. albicans* *T. mentagrophytes* *M. gypseum* *S. schenckii*	DIZ (mm) 13–31	Rojas et al. (2003)
Knema malayana Warb.	LE, SB	ME	*C. albicans*	DIZ (mm):16	Wiart et al. (2004)
Papilonaceae					
Vicia faba L.	Beans	15KDa Bowman-Birk type trypsin inhibitor	*V. mali*	IC_{50} 20 μM	Fang et al. (2010)
Phytolaccaceae					
Phytolacca tetramera Hauman	BE	Phytolaccoside B	*C. albicans* *S. cerevisiae* *C. neoformans*	74 μM 18 μM 9 μM	Escalante et al. (2008)

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Piperaceae					
Piper aducum L.	WP	ME	*C. albicans*	1.25 mg/ml	Braga et al. (2007)
Piper betle L.	LE	CE extract of the AAE	*Candida* sp.	7.81–62.5 μg/ml	Ali et al. (2010)
			Aspergillus sp.	125–500 μg/ml	
Piper crassinervium Kunth.	LE	Prenylated hydroquinones: (**1**) 1,4-dihydroxy-2-(3′,7′-dimethyl-1′-oxo-2′-*E*,6′-octadienyl)benzene (**2**) 1,4-dihydroxy-2-(3′,7′-dimethyl-1′-oxo-2′-*Z*,6′-octadienyl)benzene (**3**) 1,4-dihydroxy-2-(7′-methyl-3′-methylene-1′-oxo-4′,7′-peroxide-octyl)benzene Flavanones: (**4**) naringenin (**5**) sakuranetin	*C. cladospori* *C. sphaerospermum*	Minimum amount for inhibition of fungal growth on TLC (μg): (**1**) 1.0 (**2**) 5.0 (**3**) 5.0 (**4**) 1.0 (**5**) 1.0 (**1**) 1.0 (**2**) 10.0 (**3**) 10.0 (**4**) 5.0 (**5**) 1.0	Danelutte et al. (2003)

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Piper guineense Schum. et Thonn.	SE	AAE-AE	*M. gypseum*	MIC (μg/mL) 250	Ngono Ngane et al. (2003)
			T. mentagrophytes	125	
			T. rubrum	125	
			C. neoformans	250	
		Fraction 1 (eluted with HE)	*M. gypseum*	50	
			T. mentagrophytes	50	
			T. rubrum	100	
			A. flavus	>100	
			Scopulariopsis brevicaulis	50	
			C. albicans	>100	
			C. neoformans	100	
		Fraction 2 (eluted with HE–CHCl3/4:1)	*M. gypseum*	50	
			T. mentagrophytes	25	
			T. rubrum	25	
			A. flavus	100	
			Scopulariopsis brevicaulis	25	
			C. albicans	>100	
			C. neoformans	>100	
Piper hoffmannseggianum Schult.	LE	Isobutyl amides (hoffmannseggiamide A and B)	*C. sphaerospermum*	5.0	Marques et al. (2007)
			C. cladosporioides	5.0 μg	
Piper lanceaefolium Kunth	LE	Lanceaefolic acid methyl ester and pinocembrin chalcone	*C. albicans*	100 μg/ml	López et al. (2002)

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Piper regnellii (Miq.) CDC	LE	hydroalcoholic extract lyophilised	*C. krusei* *C. tropicalis*	125 μg/ml 500 μg/ml	Holetz et al. (2002)
Piper regnellii (Miq) CDC	LE	HA	*T. mentagrophytes; T. rubrum; M. canis; M. gypseum*	15.62; 15.62; 15.62, 62.5 μg/ml	Koroishi et al. (2008)
		Eupomatenoid-3; eupomatenoid-5	*T. rubrum*	50; 6.2 μg/ml	
Piper regnelli (Miq) CDC	–	HE fractions of HA	*Paracoccioides brasiliensis*	7.8 μg/ml	Johann et al. (2010b)
Piper scutifolium Yunck.	LE	Isobutyl amides (scutifoliamide A and B)	*Cladosporium spaherospermum* *C. cladosporioides*	5.0 μg/ml 5.0 μg/ml	Marques et al. (2007)
Poaceae					
Cymbopogon citratus (DC.) Stapf.	LE	ME	*C. krusei*	> 500 μg/ml	Tempone et al. (2008)
Polygonaceae					
Rumex nepalensis Sprengel	RO	EE	*A. flavus; A. versicolor; Fusarium moliforme; F. oxysporum; F. semitectum; Pytium* sp.; *Rhizopus* sp.; *Sporotrichum* sp.; *Thermomyces* sp.	12; 12; 12; 12; 15; 12; 10; 12; 12 μg/ml	Sharma et al. (2008)
		EAE	*Aspergillus flavus; Rhizopus* sp.	10; 10 μg/ml	
		CE	*Blastoschizomyces capitatus*	10 μg/ml	

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Primulaceae					
Primula longipes (Fretb and Subt,) Sojak	AP	AA–EE Insoluble	*C. albicans* *T. rubrum*	0.625 mg/ml 0.019 mg/ml	Buruk et al. (2006)
Proteaceae					
Lomatia hirsute Diels ex J.F. Macbr.	LE	2-Methoxy juglone	*C. albicans*	8 μg/ml	Simonsen et al. (2006)
Punicaceae					
Punica granatum L.	FR	hydroalcoholic extract lyophilised	*C. krusei* *C. parapsilosis* *C. tropicalis*	15.6 μg/ml 12.5 μg/ml 15.6 μg/ml	Holetz et al. (2002)
Punica granatum L	Peels	ME	*C. albicans* *C. glabrata* *T. rubrum* *A. niger*	0.5 mg/ml 2 mg/ml 0.125 mg/ml >2 mg/ml	Hayouni et al. (2011)
Ranunculaceae					
Clematis hirsute Per. et Guill. var. *hirsuta*	LE	80 % EE lyophilized	*C. albicans* *T. rubrum* *E. floccosum* *M. canis*	(stock dilution: 3 g fresh plant/2 ml water) ≤ 1/32	Cos et al. (2002)
Coptis chinensis	RH	–	*C. albicans*	6.25 mg/ml	Mekseepralard et al. (2010)

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Clematis tangutica (Maximowicz) Korshinsky	Aerial parts	triterpene saponins:	*Saccharomyces cerevisiae*	(**1**) (**2**): 2.5 μg/disc	Du et al. (2003)
		(**1**) 3- *O*-α- L-arabinopyranosyl hederagenin 28- *O*-α-L-rhamnopyranosyl ester	*Penicillium avellaneum*	(**1**) (**2**): 10 μg/disc	
		(**2**) 3- *O*-β- D-glucopyranosyl-(1→4)-α- L-arabinopyranosyl hederagenin 28- *O*-α-L-rhamnopyranosyl ester	*C. glabrata* *Trichosporon beigelii* *Pyricularia oryzae*	Compound (**2**) is a better antifungal agent than compound (**1**)	
Nigella sativa L.	SE	Ether (Thymoquinone)	*T. rubrum* *T. interdigitale* *T. mentagrophytes* *E. floccosum* *M. canis*	10–40 mg/ml	Aljabre et al. (2005)
Rhamnaceae					
Colubrina greggii W.	AP	EAE BE	*C. albicans*	62 μg/ml	Alanís-Garza et al. (2007)

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Ziziphus joazeiro Mart.	WP	AAE	*T. rubrum; C. guilliermondii; C. albicans; C. neoformans; F. pedrosoi*	6.5 μg/ml	Cruz et al. (2007)
Rosaceae					
Alchemilla rizeensis Buser			*Trichophyton rubrum*	0.625 mg/ml	Buruk et al. (2006)
Fragaria virginiana Duchesne	LE	AE	*Candida* sp.; C. *neoformans*	IC_{80} 50–1,600; 200 mg/ml	Webster et al. (2008)
Potentilla simplex Michx	ST, LE	AE	*Candida* sp.; *C. neoformans*	IC_{80} 25–1,600; 400 mg/ml	Webster et al. (2008)
Rubiaceae					
Saprosma fragans Beddome	AP	EE	*C. albicans*	MIC ():	Singh et al. (2006)
			C. neoformans	250 μg/ml	
			Sporothrix schenckii	125 μg/ml	
			T. mentagrophytes	500 μg/ml	
				62.5 μg/ml	
Galium mexicanum Kunth	AP	HE	*T. rubrum*	333–500 μg/ml	Bolivar et al. (2011)
			C. neoformans	333 μg/ml	
		Chloroform	*C. albicans*	666 μg/ml	
		ME	*T. rubrum*	333–500 μg/ml	
			C. neoformans	999 μg/ml	
Sommera sabicecoides K. Schum	RO	Acetylenic acids	*C. albicans*	0.8 μM	Li et al. (2008)
			Aspergillus spp.	1.1 μM	
			Trychophyton spp	0.4 μM	
Rutaceae					
Clausena anisata (WilId. Hook. F. ex Benth.	SB	ME	*C. parapsilosis*	63 μg/ml	Hamza et al. (2006)
			C. tropicalis	125 μg/ml	
			C. krusei	250 μg/ml	
			C. neoformans	250 μg/ml	

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Toddalia asiatica (L.) Lam.	LE	Isolated ulopterol	*A. flavus*	15.625 µg/ml	Karunai Raj et al. (2012)
			C. krusei	125 µg/ml	
			B. cinerea	125 µg/ml	
Salicaceae					
Salix capensis Thunb	SB	AE: 5 and 10 mg/ml	*Alternaria alternaria*	Growth inhibition (%)	Masika and Afolayan (2002)
				100.0 and 100.0	
			A. niger	6.7 and 15.0	
			Mucor hiemalis	79.0 and 100.0	
			Penicillium notatum	5.7 and 78.0	
			Schizophyllum commune	27.0 and 57.0	
		ME:0.5 and 10 mg/ml	*A. alternaria*	56.3 and 100.0	
			A. niger	66.7 and 100.0	
			M. hiemalis	100.0 and 100.0	
			P. notatum	100.0 and 100.0	
			S. commune	70.7 and 100.0	
		AAE: 5 and 10 mg/ml	*A. alternaria*	12.7 and 14.7	
			A. niger	24.7 and 23.7	
			M. hiemalis	37.7 and 56.7	
			P. notatum	1.3 and 2.3	
			S. commune	3.0 and 15.7	
		Decoction extracts	*A. alternaria*	34.7 and 59.3	
			A. niger	4.7 and 4.0	
			M. hiemalis	60.0 and 78.3	
			P. notatum	1.7 and 10.3	
			S. commune	13.3 and 33.3	

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Salvadoraceae					
Azima tetracantha Lam.	FR	ME	*C. maltosa; C. albicans; C. krusei; A. fumigatus; Absidia corymbifera; T. mentagrophytes*	Inhibition zone diameter (mm): 25; 17; 18; 16; 16; 30.	Al- Fatimi et al. (2007)
Sapindaceae					
Aesculus pavia L	Pavietin (Prenylated coumarin)	LE	*Guignardia aesculi Penicillium expansum*	20.7 µmol/l 29.9 µmol/l	Curir et al. (2007)
Dodonaea viscosa var. *angustifolia* Jacq.		AE	*C. albicans*	6.25–25 µg/ml	Patel and Coogan (2008)
Nephelium lappaceum L.	SE	Lactones	C. albicans T. mentagrophyte A. niger	DIZ (mm): 30 µg: 11–18 mm	Ragasa et al. (2005)
Saxifragaceae					
Bergenia crassifolia (L.) Fritsch	RH	EE	*C. albicans*	15.63 mg of dry plant material/ml	Kokoska et al. (2002)
Solanaceae					
Acnistus arborescens Schltdl.	LE	ME	–	–	Roumy et al. (2010)
Capsicum frutescens L. var. *fasciculatum*	SE	Monomeric lectin	*A. flavus; Fusarium moniliforme*	65, 80 %	Ngai and Ng (2007)
Cestrum auriculatum L. Heritier	LE S	95 % EE	*C. albicans* *T. mentagrophytes* *M. gypseum* *Sporothrix schenckii*	IZD (mm) 19–25	Rojas et al. (2003)

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Datura metel L.		ME	*A. fumigatus* *A. niger* *A. flavus*	0.062–1.250 mg/ml	Dabur et al. (2004)
Physalis alkekengi L.	AP Calyces	ME DM	*Candida* sp.	128–512 μg/ml 256–512 μg/ml	Helvaci et al. (2010)
Solanum chrysotrichum Schltdl.	LE	Five steroidal saponins	*T. mentagrophytes, T. rubrum, A. niger, C. albicans*	12.5–400 μg/ml	Zamilpa et al. (2002)
Solanum chrysotrichum Schltdl.		Saponins	*Candida* spp.	IC_{50}: 200–800 μg/ml	Herrera-Arellano et al. (2007)
Solanum incanum L.	FR	ME	*C. maltosa; C. albicans; C. krusei; A. fumigatus; Absidia corymbifera; T. mentagrophytes.*	Inhibition zone diameter (mm): 15; 24; 18; 25; 26; 42	Al-Fatimi et al. (2007)
Solanum xanthocarpum Schrad and Wendl.		ME	*A. fumigates* *A. niger* *A. flavus*	0.125–1.250 mg/ml	Dabur et al. (2004)
Solanum xanthocarpum Schrad and Wendl.	FR	Carpestrol; Steroidal compounds	*A. niger; Trichoderma viride*	20 μg/ml	Singh et al. (2007)
Sterculiaceae					
Hildegardia barteri (Mast.) Kosterm.	RO	Isoflavonoids/flavonoids	*C. albicans* *C. krusei* *C. glabrata* (azole resistant)	32 to >128 μg/ml	Meragelman et al. (2005)
Sterculia africana (Lour.)Fiori.	LE	ME	*C. tropicalis* *C. krusei*	63 μg/ml 250 μg/ml	Hamza et al. (2006)

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Theaceae					
Camellia sinensis (L.) Kuntze.	LE		*C. glabrata* *Clavispora lusitatiae Cryptococcus laurentii Filobasidiella neoformans* *Issatchenkia orientalis* *S. cerevisiae* *Prototheca wickerhamii*	300–4,800 μg/ml	Turchetti et al. (2005)
Theophrastaceae					
Jacquinia ruscifolia L.	FP	EE	*A. flavus; A. niger; Blastoschizomyces capitatus; Fusarium moliforme; F. oxysporum; F. semitectum; Pytium sp.; Rhizopus* sp.; *Sporotrichum* sp.; *Thermomyces* sp.	12; 12; 12; 12; 15; 15; 12; 12; 12; 12 μg/ml	Sharma et al. (2008)
		CE	*F. oxysporum*	10 μg/ml	
Umbeliferae					
Angelica sylvestris var. *stenoptera* Boiss.	LE, SE	AA–EE insol. Sol	T. rubrum	0.625 mg/ml 0.039 mg/ml	Buruk et al. (2006)
Urticaceae					
Laportea crenulata Gaud.	RO	Triterpenoid	*A. flavus; A. niger; C. albicans; Rhizopus aurizae*	30 μg/ml	Khan et al. (2007)
Verbenaceae					
Lippia adoensis Hochst. ex Walp	LE	ME	*T. mentagrophytes*	DIZ (mm): 15–22	Tadeg et al. (2005)

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Lippia alba (Mill.) N.E. Br	LE	ME	*C. krusei*	165.20 µg/ml	Tempone et al. (2008)
Lippia integrifolia (Gris.)Hieron	AP	ME	*M. canis* *E. floccosum*	500 µg/ml 250 µg/ml	Muschietti et al. (2005)
Zingiberaceae					
Boesenbergia pandurata (Roxb.) Schltr.	RH	Oil Ethanol	*C. albicans*	–	Taweechaisupapong et al. (2010)
Curcuma amarissima Roscoe	RH	Purified Elctin	*F. oxysporum* *Exserohilum turicium* *Collectrotrichum cassiicola*	Inhibition of growth at 17.5–35 µg/ml	Kheeree et al. (2010)
Curcuma zedoaria (Christm.)Roscoe *C. malabarica* K.C. Velayudhan and V:A: Amalraj and V.K. Muralidharan	RH	Extracts	*C. albicans* *A. niger*	0.01–0.47 mg/ml 0.06–0.15 mg/ml	Wilson et al. (2005)
Hedychium spicatum Lodd.		Extracts Essential oil	*Alternaria solani* *A. fumigatus* *A. flavus* *A. niger* *C. albicans* *F. oxysporum* *Mucor racemosus* *Penicillium monotricales* *P.* spp. *Rhizopus stolonifer*	DIZ (mm): 7–22	Bisht et al. (2006)

(continued)

Table 6.1 (continued)

Family/species	Plant organ	Extract	Microorganism	Effect (MIC)	Reference
Zygophyllaceae					
Balanites aegyptiaca L.	SB		*C. albicans*	125 μg/ml	Maregesi et al. (2008)
Tribulus terrestris L.		Spirostanol-based steroidal saponins	*C. albicans*	1.5–6.2 μg/ml	Bedir et al. (2002)
			C. neoformans (fluconazole resistant)		
		Spirostanol saponins	*Candida* spp.	4.4–32.4 μg/ml	Zhang et al. (2005)
			C. neoformans	10.7–42.7 μg/ml	
Tribulus terrestris L.	FR	AAE	*C. albicans*	0.15 mg/ml	Al-Bayati and Al-Mola (2008)
Zygophyllum fabago L.	WP	ME	*C. albicans*	200 μg/ml	Zaidi and Crow (2005)

Plant organ tested: *AP* aerial parts; *BR* branches; *BE* berries; *FL* flower; *FP* Flower Petals; *FR* fruit; *LE* leaves; *RH* rhizome; *RO* roots; *RS* ripe seeds; *SB* steam bark; *SE* seeds; *ST* stem; *US* unripe; *WP* whole plant; *WT* Wood of trunk.*Method*: *AWDB* Agar well-diffusion bioassay; *DD* Disc diffusion; *MD* Microbroth dilution; *PSGI* Percent spore inhibition. *Extract*: *AAE* Aqueous; *AE* Acetone; *EE* Ethano; *HA* Hydroalcoholic; *ME* Methanol; *EAE* Ethyl acetate; *CE* Chloroform; *CRE* Crude; *HE* Hexane; *BE* Butano; *DM* Dichloromethane; *C. albicans: Candida albicans*; *C. famata: Candida famata*; *C. guilliermondii: Candida guilliermondii*; *C. krusei: Candida krusei*; *C. maltosa: Candida maltosa*; *A. flavus: Aspergillus flavus*; *A. fumigatus: Aspergillus fumigatus*; *A. niger: Aspergillus niger*; *A. parasiticus: Aspergillus parasiticus*; *A. terreus: Aspergillus terreus*

6.2.2 *In vitro Methods Used to Study Antifungal Properties of Medicinal Plants*

The experimental methods usually evaluate the antifungal activity of natural products based on the inhibition of growth of several pathogenic fungi. There are several methods for detecting activity, but since they do not posses equal sensitivity or are not based upon the same principle, results will be greatly influenced by the method.

The antifungal test methods are classified into three main groups, i.e., diffusion, dilution, and bioautographic methods. It should be emphasized that many research groups have modified these methods for specific samples, such as essential oils and non-polar extracts and these small modifications make it almost impossible to directly compare results. It is, therefore, a 'must' to include at least one, but preferentially several reference compounds in each assay.

6.2.2.1 Agar-Diffusion Methods

In the diffusion technique, the tested sample at a known concentration is brought into contact with an inoculated medium and the diameter of the clear zone around it is measured at the end of the incubation period.

The tested sample is placed on the medium as filter paper discs (disc diffusion, DD), or stainless steel cylinders placed on the surface and holes punched in the medium (agar well-diffusion bioassay, AWDA). The hole-punch method is the only suitable diffusion technique for aqueous extracts, because interference by particulate matter is reduced to a minimum.

As a general rule, the diameter of fungal colonies and the percentage inhibition of the fungal growth at each tested sample are determined, taking those of the controls as 100 %. The minimum inhibitory concentration (MIC) is considered as the minimum concentration of the drug, which inhibited 80–100 % of the fungal growth.

6.2.2.2 Dilution Methods (Microbroth Dilution, MD)

To carry on dilution methods, test compounds are mixed with a suitable medium that has previously been inoculated with the test organism; this method could be developed in liquid or solid media and the microorganism growth can be measured in different ways. In the agar-dilution method, the MIC is defined as the lowest concentration able to inhibit any visible microbial growth.

In general, dilution methods are appropriate for assaying polar and non-polar extracts or compounds for determination of MIC and Minimum bactericidal/fungicidal concentration (MBC/MFC)-values. IC_{50}- and IC_{90}-values, which are the

concentrations required to produce 50 and 90 % growth inhibition, can be obtained.

The most commonly used methods are dilution, diffusion, and direct bioautography.

6.2.2.3 Bio-Autographic Methods

Bio-autography method localizes the antimicrobial activity on a chromatogram by three different approaches: (a) direct bio-autography, when microorganism growth takes place directly on the thin-layer chromatographic (TLC) plate, (b) contact bio-autography, where the tested antimicrobial compounds are transferred from the TLC plate to an inoculated agar plate through direct contact, and (c) agar overlay bio-autography, where a seeded agar medium is applied directly onto the TLC plate. This technique is not directly applicable in high capacity screening designs and its applicability is limited to microorganisms that easily grow on TLC plates.

Some works include the percent spore germination inhibition (PSGI) of medicinal herbs on spores (Rajesh 2002; Dabur et al. 2004; Mishra et al. 2010). PSGI is a simple method to evaluate the activity of new compounds against germination of fungi. Test compounds are placed in micro-culture plates at different concentrations; then, a spore's suspension is added to each plate and incubated at 37 °C for 16 h after which the number of germinated and non-germinated spores is counted using an inverted microscope. The PSGI is calculated using the following formula.

$$\text{PSGI} = 100 - \frac{\text{No. of spores germinated in treated well}}{\text{No. of spores germinated in control well}} \times 100$$

This assay is repeated three times; the lowest concentration of the compound that achieved a 97–100 % inhibition of germination is considered as MIC.

According to Cos et al. (2006), it is necessary to adopt stringent endpoint criteria in order to correct too many false-positives. For all anti-infective bioassays, IC_{50}-values should be below 100 μg/ml for mixtures and below 25 μM for pure compounds. Some microorganisms even require more severe endpoint criteria.

It must be taken into account that the infective dose may exert a profound impact on the test results. For yeasts and fungi, a satisfactory value is about 10^3 and 10^4 CFU/ml.

Several tests were carried on experimental animals such as the vaginal infection model. For this purpose, an experimental vaginal infection model (oestrogen-dependent rat vaginitis) was established. Animals were infected with *C. albicans* and assessed mycologically 3 days after infection. According to the literature, the candidal vaginitis rat model is a stable model that can be used for study of drug effect on candidal vaginitis (Pietrella et al. 2011; Yano and Fidel 2011).

Related to the published studies, the screening of herbals growing in a certain area or against a concrete fungus is the most abundant in the literature.

The most studied fungus, with a great percentage difference from the rest is *C. albicans*, followed by *A. niger* and, at great distance, a wide list of other microorganisms such as *Alternaria alternaria*, *Aspergillus flavus*, *A. fumigatus*, *A. nidulans*, *Botryodiplodia theobromae*, *Botrytis cinerea*, *Candida krusei*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata,* or *Cladosporium cladosporioides*, among others.

Most of the studied vegetal samples show a marked efficacy on the fungus growth that is represented by the MIC or IC values included in the table.

6.3 Conclusions and Future Perspectives

A thorough survey of literature confirms the suitability of herbal preparations as antifungal remedies. As already proven for many other diseases, plant kingdom is still one of the most important approaches to find new compounds that show efficacy and safety towards fungal infections.

In this sense, ethnopharmacology and ethnobotany play a crucial role as they provide with traditional knowledge on the use of herbal remedies for certain diseases. In fact, a great variety of plant species has been employed for cure of various infections; the long traditional use of these species supports their efficacy.

Its relevance is higher if we take into account the high number of biological resistances that have emerged toward synthetic antifungal medicines that are nowadays being used. The obtained information could provide with cheaper and more effective antimicrobial agents that could potentially be used to treat diseases caused by microorganisms that have developed a resistance to antibiotics.

Nonetheless, further research is needed to identify molecular targets, active compounds and structure-activity correlation, and even to prove their beneficial effects on human beings through the corresponding well-designed and performed clinical trials.

References

Abbassi F, Hani K (2012) *In vitro* antibacterial and antifungal activities of *Rhus ripartitum* used as antidiarrhoeal in Tunisian folk medicine. Nat Prod Res 26(23):2215–2218

Adekunle AA, Ikumapayi AM (2006) Antifungal property and phytochemical screening of the crude extracts of *Funtumia elastica* and *Mallotus oppositifolius*. West Indian Med J 55:219–223

Ahmad I, Fatima I (2008) Butyrylcholinesterase, lipoxygenase inhibiting and antifungal alkaloids from *Isatis tinctoria*. J Enzyme Inhib Med Chem 23:313–316

Ahmadu A, Haruna AK, Garba M, Ehinmidu JO, Sarker SD (2004) Phytochemical and antimicrobial activities of the *Daniellia oliveri* leaves. Fitoterapia 75:729–732

Aladesanmi AJ, Iwalewa EO, Adebajo AC, Akinkunmi EO, Taiwo BJ, Olorunmola FO, Lamikanra A (2006) Antimicrobial and antioxidant activities of some Nigerian medicinal plants. Afr J Trad Complement Altern Med 4:173–184

Alanís-Garza BA, González-González GM, Salazar-Aranda R, Waksman de Torres N, Rivas-Galindo VM (2007) Screening of antifungal activity of plants from the northeast of Mexico. J Ethnopharmacol 114:468–471

Al-Bayati FA, Al-Mola HF (2008) Antibacterial and antifungal activities of different parts of *Tribulus terrestris* L. growing in Iraq. J Zhejiang Univ Sci B 9:154–159

Albernaz LC, de Paula JE, Romero GA, Silva Mdo R, Grellier P, Mambu L, Espindola LS (2010) Investigation of plant extracts in traditional medicine of the Brazilian Cerrado against protozoans and yeasts. J Ethnopharmacol 131:116–121

Al-Fatimi M, Wurster M, Schröder G, Lindequist U (2007) Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. J Ethnopharmacol 111:657–666

Ali I, Khan FG, Suri KA, Gupta BD, Satti NK, Dutt P, Afrin F, Qazi GN, Khan IA (2010) *In vitro* antifungal activity of hydroxychavicol isolated from *Piper betle* L. Ann Clin Microbiol Antimicrob 9:7–15

Aljabre SH, Randhawa MA, Akhtar N, Alakloby OM, Alqurashi AM, Aldossary A (2005) Antidermatophyte activity of ether extract of *Nigella sativa* and its active principle, thymoquinone. J Ethnopharmacol 101:116–119

Altuner EM, Ceter T, Işlek C (2010) Investigation of antifungal activity of *Ononis spinosa* L. ash used for the therapy of skin infections as folk remedies. Mikrobiyol Bul 44:633–639

Amanlou M, Fazeli MR, Arvin A, Amin HG, Farsam H (2004) Antimicrobial activity of crude methanolic extract of *Satureja khuzistanica*. Fitoterapia 75:768–770

Aref HL, Salah KB, Chaumont JP, Fekih A, Aouni M, Said K (2010) In vitro antimicrobial activity of four *Ficus carica* latex fractions against resistant human pathogens (antimicrobial activity of *Ficus carica* latex). Pak J Pharm Sci 23:53–58

Ascioglu S, Rex JH, de Pauw B, Bennett JE, Bille J, Crokaert F, Denning DW, Donnelly JP, Edwards JE, Erjavec Z, Fiere D, Lortholary O, Maertens J, Meis JF, Patterson TF, Ritter J, Selleslag D, Shah PM, Stevens DA, Walsh TJ (2002) Invasive Fungal Infections Cooperative Group of the European Organization for Research and Treatment of Cancer Mycoses Study Group of the National Institute of Allergy and Infectious Diseases. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. Clin Infect Dis 34:7–14

Bagiu RV, Vlaicu B, Butnariu M (2012) Chemical composition and in vitro antifungal activity screening of the *Allium ursinum* L. (Liliaceae). Int J Mol Sci 13:1426–1436

Batawila K, Kokou K, Koumaglo K, Gbéassor M, de Foucault B, Bouchet P, Akpagana K (2005) Antifungal activities of five Combretaceae used in Togolese traditional medicine. Fitoterapia 76:264–268

Bedir E, Khan IA, Walker LA (2002) Biologically active steroidal glycosides from *Tribulus terrestres*. Pharmazie 57:491–493

Bhattacharya B, Samanta M, Pal P, Chakraborty S, Samanta A (2010) *In vitro* evaluation of antifungal and antibacterial activities of the plant *Coccinia grandis* (L.) Voight (family—Cucurbitaceae). J Phytol 2:52–57

Bisht GS, Awasthi AK, Dhole TN (2006) Antimicrobial activity of *Hedychium spicatum*. Fitoterapia 77:240–242

Bolivar P, Cruz-Paredes C, Hernández LR, Juárez ZN, Sánchez-Arreola E, Av-Gay Y, Bach H (2011) Antimicrobial, anti-inflammatory, antiparasitic, and cytotoxic activities of *Galium mexicanum*. J Ethnopharmacol 137:141–147

Bonjar GH (2004) Inhibition of Clotrimazole-resistant *Candida albicans* by plants used in Iranian folkloric medicine. Fitoterapia 75:74–76

Boonphong S, Puangsombat P, Baramee A, Mahidol C, Ruchirawat S, Kittakoop P (2007) Bioactive compounds from Bauhinia purpurea possessing antimalarial, antimycobacterial, antifungal, anti-inflammatory, and cytotoxic activities. J Nat Prod 70:795–801

Braga FG, Bouzada ML, Fabri RL, de O Matos M, Moreira FO, Scio E, Coimbra ES (2007) Antileishmanial and antifungal activity of plants used in traditional medicine in Brazil. J Ethnopharmacol 111:396–402

Brandt ME, Warnock DW (2003) Epidemiology, clinical manifestations, and therapy of infections caused by dematiaceous fungi. J Chemother 15(Suppl 2):36–47

Buruk K, Sokmen A, Aydin F, Erturk M (2006) Antimicrobial activity of some endemic plants growing in the Eastern Black Sea Region, Turkey. Fitoterapia 77:388–391

Buwa LV, van Staden J (2006) Antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South Africa. J Ethnopharmacol 103:139–142

Calderone RA (ed) (2002) Candida and candidiasis. ASM Press, Washington, DC

Canales M, Hernández T, Serrano R, Hernández LB, Duran A, Ríos V, Sigrist S, Hernández HL, Garcia AM, Angeles-López O, Fernández-Araiza MA, Avila G (2007) Antimicrobial and general toxicity activities of *Gymnosperma glutinosum*: acomparative study. J Ethnopharmacol 110:343–347

Casado R, Landa A, Calvo J, García-Mina JM, Marston A, Hostettmann K, Calvo MI (2011) Anti-inflammatory, antioxidant and antifungal activity of *Chuquiraga spinosa*. Pharm Biol 49:620–626

Céspedes CL, Avila JG, Martínez A, Serrato B, Calderón-Mugica JC, Salgado-Garciglia R (2006) Antifungal and antibacterial activities of Mexican tarragon (*Tagetes lucida*). J Agric Food Chem 54:3521–3527

Chakrabarti A, Singh R (2011) The emerging epidemiology of mould infections in developing countries. Curr Opin Infect Dis 24:521–526

Chandrasekaran M, Senthilkumar A, Venkatesalu V (2011) Antibacterial and antifungal efficacy of fatty acid methyl esters from the leaves of *Sesuvium portulacastrum* L. Eur Rev Med Pharmacol Sci 15:775–780

Chowdhury R, Hasan CM, Rashid MA (2003) Antimicrobial activity of *Toona ciliate* and *Amoora rohituka*. Fitoterapia 74:155–158

Chu KT, Ng TB (2003) Mollisin, an antifungal protein from the chestnut *Castanea mollissima*. Planta Med 69:809–813

Chu KT, Liu KH, Ng TB (2003) Cicerarin, a novel antifungal peptide from the green chickpea. Peptides 24:659–663

Cos P, Hermans N, De Bruyne T, Apers S, Sindambiwe JB, Vanden Berghe D, Pieters L, Vlietinck AJ (2002) Further evaluation of Rwandan medicinal plant extracts for their antimicrobial and antiviral activities. J Ethnopharmacol 79:155–163

Cos P, Vlietinck AJ, Berghe DV, Maes L (2006) Anti-infective potential of natural products: how to develop a stronger in vitro 'proof-of-concept'. J Ethnopharmacol 106:290–302

Cruz MC, Santos PO, Barbosa AM Jr, de Mélo DL, Alviano CS, Antoniolli AR, Alviano DS, Trindade RC (2007) Antifungal activity of Brazilian medicinal plants involved in popular treatment of mycoses. J Ethnopharmacol 111:409–412

Curir P, Galeotti F, Dolci M, Barile E, Lanzotti V (2007) Pavietin, a coumarin from *Aesculus pavia* with antifungal activity. J Nat Prod 70:1668–1671

Dabur R, Singh H, Chhillar AK, Ali M, Sharma GL (2004) Antifungal potential of Indian medicinal plants. Fitoterapia 75:389–391

Danelutte AP, Lago JH, Young MC, Kato MJ (2003) Antifungal flavanones and prenilated hydroquinones from *Piper crassinervium* Kunth. Phytochemistry 64:555–559

Daruliza KM, Yang KL, Lam KL, Priscilla JT, Sunderasan E, Ong MT (2011) Anti-*Candida albicans* activity and brine shrimp lethality test of *Hevea brasiliensis* latex B-serum. Eur Rev Med Pharmacol Sci 15:1163–1171

de Toledo CE, Britta EA, Ceole LF, Silva ER, de Mello JC, Dias Filho BP, Nakamura CV, Ueda-Nakamura T (2011) Antimicrobial and cytotoxic activities of medicinal plants of the Brazilian cerrado, using Brazilian cachaça as extractor liquid. J Ethnopharmacol 133:420–425

Deng D, Lauren DR, Cooney JM, Jensen DJ, Wurms KV, Upritchard JE, Cannon RD, Wang MZ, Li MZ (2008) Antifungal saponins from *Paris polyphylla* Smith. Planta Med 74:1397–1402

dos Santos KK, Matias EF, Tintino SR, Souza CE, Braga MF, Guedes GM, Rolón M, Vega C, de Arias AR, Costa JG, Menezes IA, Coutinho HD (2012) Cytotoxic, trypanocidal, and antifungal activities of *Eugenia jambolana* L. J Med Food 15:66–70

Du Z, Zhu N, Ze-Ren-Wang-Mu N, Shen Y (2003) Two new antifungal saponins from the Tibetan herbal medicine Clematis tangutica. Planta Med 69:547–551

Duraipandiyan V, Ignacimuthu S (2007) Antibacterial and antifungal activity of *Cassia fistula* L.: an ethnomedicinal plant. J Ethnopharmacol 112:590–594

Duraipandiyan V, Ayyanar M, Ignacimuthu S (2006) Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu. India. BMC Complement Altern Med 6:35

Duraipandiyan V, Al-Harbi NA, Ignacimuthu S, Muthukumar C (2012) Antimicrobial activity of sesquiterpene lactones isolated from traditional medicinal plant, Costus speciosus (Koen ex. Retz.) Sm. BMC Complement Altern Med 12:13

Duru ME, Oztürk M, Uğur A, Ceylan O (2004) The constituents of essential oil and *in vitro* antimicrobial activity of *Micromeria cilicica* from Turkey. J Ethnopharmacol 94:43–48

Dzoyem JP, Tangmouo JG, Lontsi D, Etoa FX, Lohoue PJ (2007) In vitro antifungal activity of extract and plumbagin from the stem bark of *Diospyros crassiflora* Hiern (Ebenaceae). Phytother Res 21:671–674

Escalante A, Gattuso M, Pérez P, Zacchino S (2008) Evidence for the mechanism of action of the antifungal phytolaccoside B isolated from *Phytolacca tetramera* Hauman. J Nat Prod 71:1720–1725

Fang EF, Hassanien AA, Wong JH, Bah CS, Soliman SS, Ng TB (2010) Purification and modes of antifungal action by *Vicia faba* cv. Egypt trypsin inhibitor. J Agric Food Chem 58:10729–10735

Feresin GE, Tapia A, Gimenez A, Gutierrez Ravelo A, Zacchino S, Sortino M, Schmeda-Hirschmann G (2003a) Constituents of the Argentinian medicinal plant *Baccharis grisebachii* and their antimicrobial activity. J Ethnopharmacol 89:73–80

Feresin GE, Tapia A, Sortino M, Zacchino S, de Arias AR, Inchausti A, Yaluff G, Rodriguez J, Theoduloz C, Schmeda-Hirschmann G (2003b) Bioactive alkyl phenols and embelin from *Oxalis arythorhiza*. J Ethnopharmacol 88:241–247

Fortún J (2011) Antifungal therapy update: new drugs and medical uses. Enferm Infecc Microbiol Clin 5(Suppl 29):38–44

Fukai T, Yonekawa M, Hou AJ, Nomura T, Sun HD, Uno J (2003) Antifungal agents from the roots of *Cudrania cochinchinensis* against *Candida*, *Cryptococcus*, and *Aspergillus* species. J Nat Prod 66:1118–1120

Fyhrquist P, Mwasumbi L, Hæggström CA, Vuorela H, Hiltunen R, Vuorela P (2002) Ethnobotanical and antimicrobial investigation on some species of *Terminalia* and *Combretum* (Combretaceae) growing in Tanzania. J Ethnopharmacol 79:169–177

Gaitán I, Paz AM, Zacchino SA, Tamayo G, Giménez A, Pinzón R, Cáceres A, Gupta MP (2011) Subcutaneous antifungal screening of Latin American plant extracts against *Sporothrix schenckii* and Fonsecaea pedrosoi. Pharm Biol 49:907–919

Ge HM, Huang B, Tan SH, da Shi H, Song YC, Tan RX (2006) Bioactive oligostilbenoids from the stem bark of *Hopea exalata*. J Nat Prod 69:1800–1802

Gertsch J, Niomawë, Gertsch-Roost K, Sticher O (2004) *Phyllanthus piscatorum*, thnopharmacological studies on a women's medicinal plant of the Yanomamï Amerindians. J Ethnopharmacol 91:181–188

Govindarajan R, Vijayakumar M, Singh M, Rao ChV, Shirwaikar A, Rawat AK, Pushpangadan P (2006) Antiulcer and antimicrobial activity of *Anogeissus latifolia*. J Ethnopharmacol 106:57–61

Hafidh RR, Abdulamir AS, Vern LS, Bakar FA, Abas F, Jahanshiri F, Sekawi Z (2011) Inhibition of growth of highly resistant bacterial and fungal pathogens by a natural product. Open Microbiol J 5:96–106

Hamza OJ, van den Bout-van den Beukel CJ, Matee MI, Moshi MJ, Mikx FH, Selemani HO, Mbwambo ZH, Van der Ven AJ, Verweij PE (2006) Antifungal activity of some Tanzanian

plants used traditionally for the treatment of fungal infections. J Ethnopharmacol 108:124–132

Hayouni EA, Miled K, Boubaker S, Bellasfar Z, Abedrabba M, Iwaski H, Oku H, Matsui T, Limam F, Hamdi M (2011) Hydroalcoholic extract based-ointment from *Punica granatum* L. peels with enhanced *in vivo* healing potential on dermal wounds. Phytomed 18:976–984

He XM, Ji N, Xiang XC, Luo P, Bao JK (2011) Purification, characterization, and molecular cloning of a novel antifungal lectin from the roots of *Ophioglossum pedunculosum*. Appl Biochem Biotechnol 165:1458–1472

Helvaci S, Kökdil G, Kawai M, Duran N, Duran G, Güvenç A (2010) Antimicrobial activity of the extracts and physalin D from *Physalis alkekengi* and evaluation of antioxidant potential of physalin D. Pharm Biol 48:142–150

Herrera-Arellano A, Martínez-Rivera Mde L, Hernández-Cruz M, López-Villegas EO, Rodríguez-Tovar AV, Alvarez L, Marquina-Bahena S, Navarro-García VM, Tortoriello J (2007) Mycological and electron microscopic study of Solanum chrysotrichum saponin SC-2 antifungal activity on *Candida* species of medical significance. Planta Med 73:1568–1573

Holetz FB, Pessini GL, Sanches NR, Garcia Cortez DA, Nakamura CV, Filho BP (2002) Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. Mem Inst Oswaldo Cruz 97:1027–1031

Hymete A, Iversen TH, Rohloff J, Erko B (2005) Screening of *Echinops ellenbeckii* and *Echinops longisetus* for biological activities and chemical constituents. Phytomedicine 12:675–679

Iranshahy M, Iranshahi M (2011) Traditional uses, phytochemistry and pharmacology of asafoetida (*Ferula assa-foetida* oleo-gum-resin)-a review. J Ethnopharmacol 134:1–10

Irshad M, Shreaz S, Manzoor N, Khan LA, Rizvi MM (2011) Anticandidal activity of *Cassia fistula* and its effect on ergosterol biosynthesis. Pharm Biol 49:727–733

Itin PH, Battegay M (2012) Skin problems in immunodeficient patients. Curr Probl Dermatol 43:9–17

Johann S, Sá NP, Lima LARS, Cisalpino PS, Cota BB, Alves TMA, Siqueira EP, Zani CL (2010a) Antifungal activity of schinol and a new biphenyl compound isolated from *Schinus terebinthifolius* against the pathogenic fungus *Paracoccidioides brasiliensis*. Ann Clin Microbiol Antimicrob 9:30

Johann S, Cisalpino PS, Watanabe GA, Cota BB, de Siqueira EP, Pizzolatti MG, Zani CL, de Resende MA (2010b) Antifungal activity of extracts of some plants used in Brazilian traditional medicine against the pathogenic fungus *Paracoccidioides brasiliensis*. Pharm Biol 48:388–396

Kariba RM, Houghton PJ, Yenesew A (2002) Antimicrobial activities of a new Schizozygane indoline alkaloid from *Schizozygia coffaeoides* and the revised structure of Isoschizogaline. J Nat Prod 65:566–569

Karkowska-Kuleta J, Rapala-Kozik M, Kozik A (2009) Fungi pathogenic to human: molecular bases of virulence of *Candida albicans*, *Cryptococcus neoformans* and *aspergillus fumigatus*. Acta Biochim Pol 56:211–224

Karunai Raj M, Balachandran C, Duraipandiyan V, Agastian P, Ignacimuthu S (2012) Antimicrobial activity of ulopterol isolated from *Toddalia asiatica* (L.) Lam.: a traditional medicinal plant. J Ethnopharmacol 140:161–165

Katkar KV, Suthar AC, Chauhan VS (2010) The chemistry, pharmacologic, and therapeutic applications of *Polyalthia longifolia*. Pharmacog Rev 4:62–68

Khan A, Haque E, Rahman MM, Mosaddik A, Rahman M, Sultana N (2007) A new triterpenoid from roots of *Laportea crenulata* and its antifungal activity. Nat Prod Res 21:959–966

Khan HU, Ali I, Khan AU, Naz R, Gilani AH (2011) Antibacterial, antifungal, antispasmodic and Ca++ antagonist effects of *Caesalpinia bonducella*. Nat Prod Res 25:444–449

Kheeree N, Sangvanich P, Puthong S, Karnchanatat A (2010) Antifungal and antiproliferative activities of lectin from the rhizomes of *Curcuma amarissima* Roscoe. Appl Biochem Biotechnol 162:912–925

Klausmeyer P, Chmurny GN, McCloud TG, Tucker KD, Shoemaker RH (2004) A novel antimicrobial indolizinium alkaloid from *Aniba panurensis*. J Nat Prod 67:1732–1735

Kokoska L, Polesny Z, Rada V, Nepovim A, Vanek T (2002) Screening of some Siberian medicinal plants for antimicrobial activity. J Ethnopharmacol 82:51–53

Koroishi AM, Foss SR, Cortez DA, Ueda-Nakamura T, Nakamura CV, Dias Filho BP (2008) *In vitro* antifungal activity of extracts and neolignans from *Piper regnellii* against dermatophytes. J Ethnopharmacol 117:270–277

Kovac-Besovic E, Duric K, Basic F, Subasic D, Imamovic J (2003) Potential antimicrobial effects of pharmacognostic drugs. Bosn J Basic Med Sci 3:16–22

Kuiate JR, Bessière JM, Zollo PH, Kuate SP (2006) Chemical composition and antidermatophytic properties of volatile fractions of hexanic extract from leaves of *Cupressus lusitanica* Mill. from Cameroon. J Ethnopharmacol 103:160–165

Lam SK, Ng TB (2002) Pananotin, a potent antifungal protein from roots of the traditional Chinese medicinal herb *Panax notoginseng*. Planta Med 68:1024–1048

Lam SK, Ng TB (2010a) Acaconin, a chitinase-like antifungal protein with cytotoxic and anti-HIV-1 reverse transcriptase activities from *Acacia confusa* seeds. Acta Biochim Pol 57:299–304

Lam SK, Ng TB (2010b) Acafusin, a dimeric antifungal protein from *Acacia confusa* seeds. Protein Pept Lett 17:817–822

Lam SK, Ng TB (2010c) Isolation and characterization of a French bean hemagglutinin with antitumor, antifungal, and anti-HIV-1 reverse transcriptase activities and an exceptionally high yield. Phytomedicine 17:457–462

Latha LY, Darah I, Jain K, Sasidharan S (2011) Effects of *Vernonia cinerea* less methanol extract on growth and morphogenesis of *Candida albicans*. Eur Rev Med Pharmacol Sci 15:543–549

Li XC, Jacob MR, Khan SI, Ashfaq MK, Babu KS, Agarwal AK, Elsohly HN, Manly SP, Clark AM (2008) Potent in vitro antifungal activities of naturally occurring acetylenic acids. Antimicrob Agents Chemother 52:2442–2448

Lin P, Xia L, Ng TB (2007) First isolation of an antifungal lipid transfer peptide from seeds of a *Brassica* species. Peptides 28:1514–1519

Lohombo-Ekomba ML, Okusa PN, Penge O, Kabongo C, Choudhary MI, Kasende OE (2004) Antibacterial, antifungal, antiplasmodial, and cytotoxic activities of *Albertisia villosa*. J Ethnopharmacol 93:331–335

López A, Ming DS, Towers N (2002) Antifungal activity of benzoic acid derivatives from *Piper lanceaefolium*. J Nat Prod 65:62–64

Mann A, Banso A, Clifford LC (2008) An antifungal property of crude plant extracts from *Anogeissus leiocarpus* and *Terminalia avicennioides*. Tanzan J Health Res 10:34–38

Maregesi SM, Pieters L, Ngassapa OD, Apers S, Vingerhoets R, Cos P, Berghe DA, Vlietinck AJ (2008) Screening of some Tanzanian medicinal plants from Bunda district for antibacterial, antifungal and antiviral activities. J Ethnopharmacol 119:58–66

Mariita RM, Orodho JA, Okemo PO, Mbugua PK (2010) Antifungal, antibacterial and antimycobacterial activity of *Entada abysinnica* Steudel ex A. Rich (Fabaceae) methanol extract. Pharmacogn Res 2:163–168

Marques JV, Kitamura RO, Lago JH, Young MC, Guimarães EF, Kato MJ (2007) Antifungal amides from *Piper scutifolium* and *Piper hoffmanseggianum*. J Nat Prod 70:2036–2039

Masika PJ, Afolayan AJ (2002) Antimicrobial activity of some plants used for the treatment of livestock disease in the Eastern Cape, South Africa. J Ethnopharmacol 83:129–134

Masoko P, Picard J, Eloff JN (2005) Antifungal activities of six South African *Terminalia* species (Combretaceae). J Ethnopharmacol 99:301–308

Mekseepralard C, Kamkaen N, Wilkinson JM (2010) Antimicrobial and antioxidant activities of traditional Thai herbal remedies for aphthous ulcers. Phytother Res 24:1514–1519

Mensah AY, Houghton PJ, Dickson RA, Fleischer TC, Heinrich M, Bremner P (2006) *In vitro* evaluation of effects of two Ghanaian plants relevant to wound healing. Phytother Res 20:941–944

Meragelman TL, Tucker KD, McCloud TG, Cardellina JH 2nd, Shoemaker RH (2005) Antifungal flavonoids from Hildegardia barteri. J Nat Prod 68:1790–1792

Mishra BB, Kishore N, Tiwari VK, Singh DD, Tripathi V (2010) A novel antifungal anthraquinone from seeds of *Aegle marmelos* Correa (family Rutaceae). Fitoterapia 81:104–107

Moshi MJ, Mbwambo ZH (2005) Some pharmacological properties of extracts of *Terminalia sericea* roots. J Ethnopharmacol 97:43–47

Mothana RA, Lindequist U (2005) Antimicrobial activity of some medicinal plants of the island Soqotra. J Ethnopharmacol 96:177–181

Muhammad I, Li XC, Jacob MR, Tekwani BL, Dunbar DC, Ferreira D (2003) Antimicrobial and antiparasitic (+)-*trans*-hexahydrodibenzopyrans and analogues from *Machaerium multiflorum*. J Nat Prod 66:804–809

Muschietti L, Derita M, Sülsen V, de Dios Muñoz J, Ferraro G, Zacchino S, Martino V (2005) *In vitro* antifungal assay of traditional Argentine medicinal plants. J Ethnopharmacol 102:233–238

Navarro García VM, González A, Fuentes M, Aviles M, Ríos MY, Zepeda G, Rojas MG (2003) Antifungal activities of nine tradicional Mexican medicinal plants. J Ethnopharmacol 87:85–88

Ncube B, Ngunge VN, Finnie JF, Van Staden J (2011) A comparative study of the antimicrobial and phytochemical properties between outdoor grown and micropropagated *Tulbaghia violacea* Harv. plants. J Ethnopharmacol 134:775–780

Ngai PH, Ng TB (2007) A lectin with antifungal and mitogenic activities from red cluster pepper (*Capsicum frutescens*) seeds. Appl Microbiol Biotechnol 74:366–371

Ngono Ngane A, Biyiti L, Bouchet P, Nkengfackd A, Amvam Zolloa PH (2003) Antifungal activity of *Piper guineense* of Cameroon. Fitoterapia 74:464–468

Ngono Ngane A, Ebelle Etame R, Ndifor F, Biyiti L, Amvam Zollo PH, Bouchet P (2006) Antifungal activity of *Chromolaena odorata* (L.) King and Robinson (Asteraceae) of Cameroon. Chemotherapy 52:103–106

Oberlies NH, Burgess JP, Navarro HA, Pinos RE, Fairchild CR, Peterson RW, Soejarto DD, Farnsworth NR, Kinghorn AD, Wani MC, Wall ME (2002) Novel bioactive clerodane diterpenoids from the leaves and twigs of *Casearia sylvestris*. J Nat Prod 65:95–99

Omer ME, Elnima EI (2003) Antimicrobial activity of *Ximenia americana*. Fitoterapia 74:122–126

Ouattara B, Kra AM, Coulibaly A, Guede-Guina F (2007) Efficiency of ethanol of *Thonningia sanguinea* against *Cryptococcus neoformans*. Sante 17:219–222

Patel M, Coogan MM (2008) Antifungal activity of the plant *Dodonaea viscosa* var. *angustifolia* on *Candida albicans* from HIV-infected patients. J Ethnopharmacol 118:173–176

Pemán J, Salavert M (2012) General epidemiology of invasive fungal disease. Enferm Infecc Microbiol Clin 30:90–98

Pérez-Laínez D, García-Mateos R, San Miguel-Chávez R, Soto-Hernández M, Rodríguez-Pérez E, Kite G (2008) Bactericidal and fungicidal activities of *Calia secundiflora* (Ort.) Yakovlev. Z Naturforsch C 63:653–657

Pettit GR, Zhang Q, Pinilla V, Hoffmann H, Knight JC, Doubek DL, Chapuis JC, Pettit RK, Schmidt JM (2005) Antineoplastic agents. 534. isolation and structure of sansevistatins 1 and 2 from the African *Sansevieria ehrenbergii*. J Nat Prod 68:729–733

Pfaller MA (2012) Antifungal drug resistance: mechanisms, epidemiology, and consequences for treatment. Am J Med 125(1 Suppl):S3–S13

Pietrella D, Angiolella L, Vavala E, Rachini A, Mondello F, Ragno R, Bistoni F, Vecchiarelli A (2011) Beneficial effect of *Mentha suaveolens* essential oil in the treatment of vaginal candidiasis assessed by real-time monitoring of infection. BMC Complement Altern Med 11:18

Pinto E, Pina-Vaz C, Salgueiro L, Gonçalves MJ, Costa-de-Oliveira S, Cavaleiro C, Palmeira A, Rodrigues A, Martinez-de-Oliveira J (2006) Antifungal activity of the essential oil of *Thymus pulegioides* on *Candida*, *Aspergillus* and dermatophyte species. J Med Microbiol 55:1367–1373

Plaza A, Cinco M, Tubaro A, Pizza C, Piacente S (2003) New triterpene glycosides from the stems of *Anomospermum grandifolium*. J Nat Prod 66:1606–1610

Ragasa CY, de Luna RD, Cruz WC Jr, Rideout JA (2005) Monoterpene lactones from the seeds of *Nephelium lappaceum*. J Nat Prod 68:1394–1396

Rajesh GLS (2002) Studies on antimycotic properties of *Datura metel*. J Ethnopharmacol 80:193–197

Rios MY, Aguilar-Guadarrama AB, Navarro V (2003) Two new benzofuranes from *Eupatorium aschenbornianum* and their antimicrobial activity. Planta Med 69:967–970

Rojas R, Bustamante B, Bauer J, Fernández I, Albán J, Lock O (2003) Antimicrobial activity of selected Peruvian medicinal plants. J Ethnopharmacol 88(2–3):199–204

Rotta I, Sanchez A, Gonçalves PR, Otuki MF, Correr CJ (2012) Efficacy and safety of topical antifungals in the treatment of dermatomycosis: a systematic review. Br J Dermatol 166:927–933

Roumy V, Biabiany M, Hennebelle T, el Aliouat M, Pottier M, Joseph H, Joha S, Quesnel B, Alkhatib R, Sahpaz S, Bailleul F (2010) Antifungal and cytotoxic activity of withanolides from *Acnistus arborescens*. J Nat Prod 73:1313–1317

Rukayadi Y, Shim JS, Hwang JK (2008) Screening of Thai medicinal plants for anticandidal activity. Mycoses 51:308–312

Santos KK, Matias EF, Sobral-Souza CE, Tintino SR, Morais-Braga MF, Guedes GM, Santos FA, Sousa AC, Rolón M, Vega C, de Arias AR, Costa JG, Menezes IR, Coutinho HD (2012) Trypanocide, cytotoxic, and antifungal activities of *Momordica charantia*. Pharm Biol 50:162–166

Sartori MR, Pretto JB, Cruz AB, Bresciani LF, Yunes RA, Sortino M, Zacchino SA, Cechinel VF (2003) Antifungal activity of fractions and two pure compounds of flowers from *Wedelia paludosa* (*Acmela brasiliensis*) (Asteraceae). Pharmazie 58:567–569

Sasaki K, Abe H, Yoshizaki F (2002) *In vitro* antifungal activity of naphtoquinone derivatives. Biol Pharm Bull 25:669–670

Sasidharan S, Darah I, Jain K (2011) In vitro and in situ antiyeast activity of *Gracilaria changii* methanol extract against Candida albicans. Eur Rev Med Pharmacol Sci 15:1020–1026

Sathiamoorthy B, Gupta P, Kumar M, Chaturvedi AK, Shukla PK, Maurya R (2007) New antifungal flavonoid glycoside from *Vitex negundo*. Bioorg Med Chem Lett 17:239–242

Sautour M, Miyamoto T, Lacaille-Dubois MA (2005) Steroidal saponins from *Smilax medica* and their antifungal activity. J Nat Prod 68:1489–1493

Schmourlo G, Mendonça-Filho RR, Alviano CS, Costa SS (2005) Screening of antifungal agents using ethanol precipitation and bioautography of medicinal and food plants. J Ethnopharmacol 96:563–568

Shai LJ, McGaw LJ, Aderogba MA, Mdee LK, Eloff JN (2008) Four pentacyclic triterpenoids with antifungal and antibacterial activity from *Curtisia dentata* (Burm.f) C.A. Sm. leaves. J Ethnopharmacol 119:238–244

Sharma RS, Mishra V, Singh R, Seth N, Babu CR (2008) Antifungal activity of some Himalayan medicinal plants and cultivated ornamental species. Fitoterapia 79:589–591

Silva O, Gomes ET (2003) Guieranone A, a naphthyl butenone from the leaves of *Guiera senegalensis* with antifungal activity. J Nat Prod 66:447–449

Simonsen HT, Adsersen A, Berthelsen L, Christensen SB, Guzmán A, Mølgaard P (2006) Ethnopharmacological evaluation of radal (leaves of *Lomatia hirsuta*) and isolation of 2-methoxyjuglone. BMC Complement Altern Med 6:29

Singh DN, Verma N, Raghuwanshi S, Shukla PK, Kulshreshtha DK (2006) Antifungal anthraquinones from *Saprosma fragrans*. Bioorg Med Chem Lett 16:4512–4514

Singh OM, Subharani K, Singh NI, Devi NB, Nevidita L (2007) Isolation of steroidal glycosides from *Solanum xanthocarpum* and studies on their antifungal activities. Nat Prod Res 21:585–590

Singh DN, Verma N, Raghuwanshi S, Shukla PK, Kulshreshtha DK (2008) Antifungal activity of *Agapanthus africanu*s extractives. Fitoterapia 79:298–300

Singh B, Chopra A, Ishar MPS, Sharma A, Raj T (2010) Pharmacognostic and antifungal investigations of *Elaeocarpus ganitrus* (Rudrakasha). Indian J Pharm Sci 72:261–265

Singh D, Sharma U, Kumar P, Gupta YK, Dobhal MP, Singh S (2011) Antifungal activity of plumericin and isoplumericin. Nat Prod Commun 6:1567–1568

Siritapetawee J, Thammasirirak S, Samosornsuk W (2012) Antimicrobial activity of a 48-kDa protease (AMP48) from *Artocarpus heterophyllus* latex. Eur Rev Med Pharmacol Sci 16:132–137

Sobel JD (2007) Vulvovaginal candidosis. Lancet 369:1961–1971

Stein AC, Sortino M, Avancini C, Zacchino S, von Poser G (2005) Ethnoveterinary medicine in the search for antimicrobial agents: antifungal activity of some species of *Pterocaulon* (Asteraceae). J Ethnopharmacol 99:211–214

Tadeg H, Mohammed E, Asres K, Gebre-Mariam T (2005) Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders. J Ethnopharmacol 100:168–175

Taweechaisupapong S, Singhara S, Lertsatitthanakorn P, Khunkitti W (2010) Antimicrobial effects of *Boesenbergia pandurata* and *Piper sarmentosum* leaf extracts on planktonic cells and biofilm of oral pathogens. Pak J Pharm Sci 23:224–231

Tempone AG, Sartorelli P, Teixeira D, Prado FO, Calixto IA, Lorenzi H, Melhem MS (2008) Brazilian flora extracts as source of novel antileishmanial and antifungal compounds. Mem Inst Oswaldo Cruz 103:443–449

Tian J, Shen Y, Yang X, Liang S, Shan L, Li H, Liu R, Zhang W (2010) Antifungal cyclic peptides from *Psammosilene tunicoides*. J Nat Prod 73:1987–1992

Torey A, Sasidharan S (2011) Anti-*Candida albicans* biofilm activity by *Cassia spectabilis* standardized methanol extract: an ultrastructural study. Eur Rev Med Pharmacol Sci 15:875–882

Turchetti B, Pinelli P, Buzzini P, Romani A, Heimler D, Franconi F, Martini A (2005) *In vitro* antimycotic activity of some plant extracts towards yeast and yeast-like strains. Phytother Res 19:44–49

Valdés AF, Martínez JM, Lizama RS, Vermeersch M, Cos P, Maes L (2008) *In vitro* antimicrobial activity of the Cuban medicinal plants *Simarouba glauca* DC, *Melaleuca leucadendron* L and *Artemisia absinthium* L. Mem Inst Oswaldo Cruz 103:615–618

Volleková A, Kost'álová D, Kettmann V, Tóth J (2003) Antifungal activity of *Mahonia aquifolium* extract and its major protoberberine alkaloids. Phytother Res 17:834–837

Walsh TJ, Anaissie EJ, Denning DW et al (2008) Treatment of aspergillosis: clinical practice guidelines of the infectious diseases society of America. Clin Infect Dis 46:327–360

Wang Q, Li F, Zhang X, Zhang Y, Hou Y, Zhang S, Wu Z (2011) Purification and characterization of a CkTLP protein from *Cynanchum komarovii* seeds that confers antifungal activity. PLoS ONE 6:e16930

Webster D, Taschereau P, Belland RJ, Sand C, Rennie RP (2008) Antifungal activity of medicinal plant extracts; preliminary screening studies. J Ethnopharmacol 115:140–146

Wiart C, Mogana S, Khalifah S, Mahan M, Ismail S, Buckle M, Narayana AK, Sulaiman M (2004) Antimicrobial screening of plants used for traditional medicine in the state of Perak, Peninsular Malaysia. Fitoterapia 75:68–73

Wilson B, Abraham G, Manju VS, Mathew M, Vimala B, Sundaresan S, Nambisan B (2005) Antimicrobial activity of *Curcuma zedoaria* and *Curcuma malabarica* tubers. J Ethnopharmacol 99:147–151

Woldemichael GM, Wink M (2002) Triterpene glycosides of *Lupinus angustifolius*. Phytochemistry 60:323–327

Wong JH, Ng TB (2003) Gymnin, a potent defensin-like antifungal peptide from the Yunnan bean (*Gymnocladus chinensis* Baill). Peptides 24:963–968

Wuthi-udomlert M, Prathanturarug S, Wongkrajang Y (2002) Antifungal activity and local toxicity study of *Alangium salviifolium* subsp. *hexapetalum*. Southeast Asian J Trop Med Public Health 33(Suppl 3):152–154

Yamaguchi MU, Garcia FP, Cortez DA, Ueda-Nakamura T, Filho BP, Nakamura CV (2011) Antifungal effects of Ellagitannin isolated from leaves of *Ocotea odorifera* (Lauraceae). Antonie Van Leeuwenhoek 99:507–514

Yano J, Fidel PL Jr (2011) Protocols for vaginal inoculation and sample collection in the experimental mouse model of *Candida* vaginitis. J Vis Exp (58):e3382. doi: 10.3791/3382

Zaidi MA, Crow SA Jr (2005) Biologically active traditional medicinal herbs from Balochistan, Pakistan. J Ethnopharmacol 96:331–334

Zamilpa A, Tortoriello J, Navarro V, Delgado G, Alvarez L (2002) Five new steroidal saponins from *Solanum chrysotrichum* leaves and their antimycotic activity. J Nat Prod 65:1815–1819

Zhang JD, Cao YB, Xu Z, Sun HH, An MM, Yan L, Chen HS, Gao PH, Wang Y, Jia XM, Jiang YY (2005) In vitro and in vivo antifungal activities of the eight steroid saponins from *Tribulus terrestris* L. with potent activity against fluconazole-resistant fungal pathogens. Biol Pharm Bull 28:2211–2215

Zhao M, Ma Y, Pan YH, Zhang CH, Yuan WX (2011) A hevein-like protein and a class I chitinase with antifungal activity from leaves of the paper mulberry. Biomed Chromatogr 25:908–912

Zhou L, Li D, Jiang W, Qin Z, Zhao S, Qiu M, Wu J (2007) Two ellagic acid glycosides from *Gleditsia sinensis* Lam. with antifungal activity on Magnaporthe grisea. Nat Prod Res 21:303–309

Chapter 7
Recent Progress in Research on Plant Antifungal Proteins: A Review

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Abstract The intent of this article is to review plant antifungal proteins that have been purified and characterized in the last 5–6 years. The antifungal proteins reported encompass 2S albumins, amidases and ureases, chitinases, defensins and defensin-like peptides, beta-1, 3-glucanases, beta-lactoglobulin-like protein, lectins, lipid transfer proteins, peroxidases, proteases, protease inhibitors, ribonucleases, thaumatin-like proteins, and other antifungal proteins. These proteins demonstrate a diversity of structures. Some of them possess additional activities such as antiproliferative activity toward cancer cells, inhibitory activity toward viral enzymes, and specific aforementioned enzyme activities. The antifungal mechanism may involve permeabilization of fungal cell membrane, induction of reactive oxygen species, and triggering of apoptosis.

7.1 Introduction

Antimicrobial proteins and peptides with potent antifungal, antibacterial, and antiviral activities contribute significantly to the innate host defense mechanisms of most living organisms. The emergence of microbes recalcitrant to currently deployed antibiotics has motivated researchers to hunt for new antimicrobial proteins and examine their modes of action. Genes encoding chitinase, chitinase-like protein, beta-1, 3-glucanase, the ribosome inactivating protein trichosanthin, *Aspergillus giganteus* antifungal protein, synthetically prepared antifungal genes

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Ap-CecA and ER-CecA, N-terminally-modified antimicrobial cationic peptide temporin-A, and mustard defensin have been employed for developing fungi-resistant transgenic millet, potato, tobacco, and peanut plants (Ceasar and Ignacimuthu 2012).

Besides antifungal proteins, there are nonpeptidic molecules with antifungal activity. Glyceollin is one of the major phytoalexins and phytoestrogens and an important prenylflavonoid in soybean. It exhibits antifungal activity (toward *Fusarium solani, Phakospora pachyrhizi, Diaporthe phaseolorum, Macrophomina phaseolina, Sclerotina sclerotiorum, Phytophthora sojae, Cercospora sojina, Phialophora gregata,* and *Rhizoctonia solani).* Hence, plants have an armamentarium of peptidic as well as nonpeptidic compounds to defend themselves against fungal pathogens.

Plants produce a host of proteins involved in the defense against pathogens and invading organisms, one of which is protease inhibitor. Plant protease inhibitors inhibit the proliferation of pathogenic fungi and bacteria and represent candidates for use as lead compounds for the development of new antimicrobials (Kim et al. 2009). Besides protease inhibitors, there is a sizeable number of other plant antifungal proteins that contribute to the defense against fungal pathogens. The variety of antifungal proteins and peptides reported in recent years are reviewed in the following sections.

7.2 Antifungal Proteins

7.2.1 2S Albumins

Five isoforms of an antifungal protein from dandelion (*Taraxacum officinale*) seeds, including To-A1, which exhibited N-terminal amino acid sequence homology to sunflower 2S albumin, inhibited phytopathogenic fungi and the oomycete *Phytophthora infestans* at micromolar concentrations (Odintsova et al. 2010).

7.2.2 Amidases and Ureases

A 60 kDa antifungal amidase with an N-terminal amino acid sequence closely resembling those of amidases was purified from *Peltophorum pterocarpum* seeds. It exhibited amidase activity and hydrolyzed iodoacetamide, acrylamide, and urea. Its optimum pH and temperature were at pH 9 and 50 °C, respectively. It also inhibited mycelial growth in *Rhizoctonia solani* and HIV-1 reverse transcriptase with an IC_{50} of 0.65 μM and 27 nM, respectively. Congo red staining after

incubation with peltopterin disclosed chitin deposition at hyphal tips in *R. solani*. Its antifungal activity was pH-stable and thermostable. Lam and Ng 2010a).

Soybean (*Glycine max*) and jack bean (*Canavalia ensiformis*) ureases exhibited antifungal and insecticidal activities. Cotton (*Gossypium hirsutum*) seed urease displayed potent antifungal properties despite low ureolytic activity. Like other ureases, the antifungal effect of cotton urease was preserved even following exposure to an irreversible inhibitor of its urease activity (Menegassi et al. 2008).

7.2.3 Chitinases

A 3,184 Da protease-stable and thermostable antifungal peptide from *Amaranthus hypochondriacus* seeds, Ay-AMP, was a chitin-binding and chitinolytic protein with a single cysteine/glycine-rich chitin-binding domain. It inhibits various fungal pathogens, such as *Aspergillus candidus, A. ochraceus, Alternaria alternata, Candida albicans, F. solani, Geotrichum candidum, Penicillium chrysogenum,* and *Trichoderma sp.* (Rivillas-Acevedo and Soriano-García 2007).

Two 30 kDa proteins from fruits of the emperor banana, with N-terminal sequence homology to chitinases, hampered mycelial growth in *Fusarium oxysporum* but not in *Mycosphaerella arachidicola*. The protein more strongly bound on Mono S had a higher antifungal activity toward *F. oxysporum* than the less strongly bound protein (Ho and Ng 2007).

A 30.4 kDa chitinase-like protein from peanut (*Arachis hypogaea*) seeds with both antifungal and antibacterial activities exerted potent antifungal action toward a variety of fungal species, including *A. alternata, Botrytis cinerea,, F. solani, F. oxysporum, Physalospora piricola,* and *Pythium aphanidermatum.* It also exhibited antiproliferative activity against tumor cells (Wang et al. 2007).

A class III chitinase cDNA (BoChi3-1) was cloned using a cDNA library from suspension-cultured bamboo *(Bambusa oldhamii)* cells and then transformed into yeast (*Pichia pastoris* X-33) for expression. A recombinant 28.3 kDa chitinase together with another recombinant 35.7 kDa chitinase were purified from the yeast's culture broth. Both recombinant chitinases were encoded by BoChi3-1. The 35.7 kDa isoform but not the 28.3 kDa isoform was glycosylated. The optimal pH values and optimal temperatures for hydrolysis of ethylene glycol chitin (EGC), were pH 3 and pH 4, and 80 and 70 °C, for 35.7 and 28.3 kDa, isoforms, respectively. The K_m values were 1.35 and 0.65 mg/ml, respectively. EGC hydrolysis catalyzed by the 35.7 kDa isoforms was more efficient than that catalyzed by the 28.3 kDa isoform. Both recombinant BoCHI3-1 isoforms inhibited *Scolecobasidium longiphorum* and displayed remarkable thermal stability (up to 70 °C) and storage stability (up to a year at 4 °C) (Kuo et al. 2008).

Recombinant rice chitinase purified from *P. pastoris* exhibited different antifungal activities against the fungi *Aspergillus niger*, *Botrytis squamosa*, *P. aphanidermatum*, and *Rhizopus stolonifer*. There was a direct correlation to the

surface microstructure and the proportion of chitin in the fungal cell wall as shown by scanning electron microscopy and Fourier transform infrared spectroscopy. The findings provide an insight to the mechanism of antifungal action of the recombinant chitinase and the prospects of its application to crop protection and post-harvest storage of fruits and vegetables (Yan et al. 2008).

A 30.6 kDa monomeric antifungal chitinase with a pI of 7.6 was isolated from the Canadian cranberry beans (*Phaseolus vulgaris*). The optimum temperature and optimum pH for activity toward the substrate N-acetyl-glucosamine was 40–55 °C and 5.4, respectively. The enzyme potently inhibited *B. cinerea, F. oxysporum, P. piricola,* and *P. aphanidermatum*. The chitinase and antifungal activities were stable up to 58 °C (Wang et al. 2009b).

A 32 kDa chitinase-like antifungal protein from *Acacia confusa* seeds, designated as acaconin, possessed an N-terminal sequence with pronounced homology to chitinases, but devoid of chitinase activity. It inhibited mycelial growth in *R. solani* with an IC_{50} of 30 ± 4 μM. The antifungal activity was unaffected when the ambient pH fluctuated between pH 4 and pH 10 and when the temperature changed from 0 to 70 °C. Congo Red staining at the tips of *R. solani* hyphae signified inhibition of fungal growth. In contrast, no inhibitory activity toward *M. arachidicola, F. oxysporum, Helminthosporium maydis,* and *Valsa mali* could be discerned. Acaconin inhibited proliferation of breast cancer MCF-7 cells with an IC_{50} of 128 ± 9 μM, but there was no effect on hepatoma HepG2 cells. Its IC_{50} value toward HIV-1 reverse transcriptase was 10 ± 2.3 μM. The unique features of acaconin encompass specific antifungal and antitumor actions, potent HIV-reverse transcriptase inhibitory activity, and relatively high stability when subjected to variations in ambient pH and temperature (Lam and Ng 2011a).

Two proteins (a 18.8 kDa hevein-like protein PMAPI and a 31 kDa class I chitinase PMAPII) with activity against *Trichoderma viride* were obtained from paper mulberry *(Broussonetia papyrifera*) leaves. They both had an IC_{50} of 0.1 μg/μl against *T. viride* (Zhao et al. 2011).

7.2.4 Defensins and Defensin-Like Peptides

Plant defensins are antimicrobial peptides with an abundance of cysteine residues, and analogous of three-dimensional structures. They are composed of an α-helix and three anti-parallel β-strands stabilized by four disulfide bonds. In one investigation, small putative proteins containing eight cysteines and with a molecular mass below 10 kDa were screened based on the sugarcane expressed sequence tag base. Open reading frames that demonstrated 25–100 % similarity in amino acid sequence with other defensins in the NCBI database and possessing eight cysteines were chosen. This resemblance is adequate for the purpose of folding prediction, but not good enough for inference of biological activity. Six putative defensins (Sd1–6) were selected, and activity assays showed that recombinant Sd1, Sd3, and Sd5 expressed antifungal but not antibacterial activity. Structural characterization, based on

circular dichroism and nuclear magnetic resonance spectroscopy, disclosed that the structures of these Sds were compatible with alpha/beta proteins, a characteristic anticipated for plant defensins. Phylogenetic analysis indicated that sugarcane defensins could be classified within defensins from the Poaceae family and the Andropogoneae tribe. The study shows that defensins manifest high conservation in the Poaceae family and that a similar conservation may be encountered in other families. Evolutionary relationships within plant families can be employed to predict and annotate new defensins in genomes and group them in evolutionary classes to facilitate explorations of their biological role (De-Paula et al. 2008).

RsAFP2 (*Raphanus sativus* antifungal peptide 2) is a plant defensin that is nontoxic to mammalian cells and has prophylactic efficacy against murine candidiasis. It inhibits *C. albicans* and other *Candida* species. It interacts with fungal glucosylceramides. Glucosylceramide levels in *Candida* species exhibit a correlation with RsAFP2 sensitivity (Tavares et al. 2008). RsAFP2 interacts with glucosylceramides (GlcCer) in membranes of susceptible fungi and yeast to bring about membrane permeabilization and subsequently death. However, permeabilization due to insertion of RsAFP2 in carboxyfluorescein-containing small unilamellar vesicles containing purified GlcCer could not be demonstrated. RsAFP2 elicits production of reactive oxygen species (ROS) in wild-type *C. albicans*, but did not affect an RsAFP2-resistant Deltagcs *C. albicans* mutant deficient in the membrane RsAFP2- binding site. Upstream binding of RsAFP2 to GlcCer is requisite for reactive oxygen species production culminating in yeast cell death. Both ROS generation and inhibition of fungal growth caused by RsAFP2 are undermined by the antioxidant ascorbic acid. The findings suggest the presence of an intracellular plant defensin-triggered signaling cascade involving ROS formation and resulting in arrest of fungal growth (Aerts et al. 2007).

Sequence analysis of the cloned cDNA, defensin from *P. vulgaris* cv. Pérola seeds (PVD1), demonstrated 314 bp encoding a polypeptide composed of 47 amino acid residues. The deduced peptide shows high homology to defensins from *Cicer arietinum* (95 % identity), *Vigna unguiculata* (93 % identity), *and Pachyrhizus erosus* (87 % identity). PvD1 hampered growth in the yeasts, *C. albicans, C. guilliermondii, C. parapsilosis, C. tropicalis, Kluyveromyces marxiannus,* and *Saccharomyces cerevisiae*. PvD1 also inhibited the phytopathogenic fungi *Fusarium lateritium, F. oxysporum, F. solani, and Rizoctonia solani* (Games et al. 2008).

A berry-specific cDNA sequence designated as *Vitis vinifera* antimicrobial peptide 1 (Vv-AMP1) was isolated from *V. vinifera*. Vv-AMP1 encodes a peptide with sequence resemblance to plant defensins and only expressed in berry tissue since the onset of berry ripening. Recombinant Vv-AMP1, which exhibited a molecular mass of 5.495 kDa, manifested pronounced thermostability and potent antifungal activity against a diversity of fungal phytopathogens including *F. oxysporum* and *Verticillium dahliae*. Vv-AMP1 potently suppressed hyphal elongation. Its antifungal activity might be attributed to permeabilization of the fungal membranes as evidenced by results of the propidium iodide uptake assay (de Beer and Vivier 2008).

Plant defensins are small (45–54 amino acids), highly basic, and cysteine-rich peptides structurally related to defensins of other organisms, including insects and mammals. The antifungal activity of the 6 kDa defensin-like antifungal peptide from French bean (*P. vulgaris*) seeds was stable in the temperature range of 0–90 °C for 20 min, in the pH range of 4–10, and following treatment with trypsin (1 mg/ml) at 37 °C for 1 h (Leung et al. 2008).

An 7.3 kDa defensin-like antifungal peptide from dried "Cloud Bean" (*P. vulgaris*) seeds inhibited the fungi *F. oxysporum* and *M. arachidicola* and proliferation of L1210 mouse leukemia cells and MBL2 lymphoma cells with an IC_{50} of 2.2, 1.8, 10 and 40 μM, respectively (Wu et al. 2011)

A 7.1 kDa defensin-like antifungal peptide from dried Nepalese large red beans (*Phaseolus angularis*) inhibited mycelial growth in *M. arachidicola* and *F. oxysporum* with an IC_{50} value of 1.8 and 1.4 μM, respectively. It exerted an antiproliferative action on L1210 leukemia cells and MBL2 lymphoma cells with an IC_{50} of 15 and 60 μM, respectively (Ma et al. 2009).

The cloned plant defensin corn 1 (Pdc1) gene expressed in *P. pastoris* was more efficacious than the same gene expressed in *E. coli* in inhibiting growth of *Fusarium graminearum*. FTIR analysis disclosed that the more active protein possessed more beta-sheets and less random unordered structure. Elimination of the His-tag employed for protein purification augmented the antifungal activity (Kant et al. 2009).

A 6.8 kDa moderately thermostable (up to 80 °C) defensin-like peptide from the large lima bean *Phaseolus limensis* designated as limyin, impeded mycelial growth in *A. alternata, B. cinerea,* and *F. solani*. It showed antiproliferative activity toward Bel-7402 human hepatoma cells and SHSY5Y neuroblastoma cells, although there was no effect on the bacteria *Staphylococcus aureus* and *Salmonella* (Wang et al. 2009a).

Recombinant, cysteine-rich plant proteins snakin-1 (SN1) and defensin (PTH1) potently inhibited the phytopathogenic fungi *Colletotrichum coccoides* and *B. cinerea* (IC_{50} 5–14 μM) and the phytopathogenic bacterium *Clavibacter michiganensis* subsp. sepedonicus (IC_{50} 1.5–8 μM). However, *Pseudomonas syringae* pv. syringae and *P. syringae* pv. Tabaci were inhibited with an attenuated potency. A pronounced synergistic antimicrobial effect against *P. syringae* pv. syringae SN1 and PTH1 in conjunction produced an additive effect against *P. syringae* pv. tabaci. SN1 alone or in combination with SN1 caused *C. michiganensis* cells to undergo aggregation (Kovalskaya and Hammond 2009).

Two highly homologous defensins, Sm-AMP-D1 and Sm-AMP-D2, from common chickweed (*Stellaria media*) seeds inhibited phytopathogenic fungi and oomycetes in the micromolar range (Slavokhotova et al. 2011).

The 5,601 Da *Pinus sylvestris* defensin 1 demonstrated antifungal potency against an array of fungal pathogens. An escalated level of defensin is detected in Scots pine seedlings during germination and infection by the pathogen *Heterobasidion annosum* (Kovaleva et al. 2011).

Plant defensins MsDef1 and MtDef4 potently suppressed the growth of several filamentous fungi including *F. graminearum.* Both defensins own a highly

conserved γ-core motif (GXCX(3-9)C), composed of β2 and β3 strands and the interposed loop, which is a salient feature of disulfide-stabilized antimicrobial peptides. Nevertheless, significant differences in amino acid sequence and in net charge appear in the γ-core motifs of these two defensins. The chief determinants of the antifungal activity and morphogenicity of MsDef1 and MtDef4 lie in the γ-core motifs. MsDef1 but not MtDef4 induced extensive hyperbranching of fungal hyphae. The MsDef1-γ4 variant with the γ-core motif of MsDef1 substituted by that of MtDef4 evinced a potency similar to MtDef4 and was also incapable of stimulating hyphal hyperbranching. The γ-core motif of MtDef4 alone but not that of MsDef1was capable of inhibiting fungal growth. The cationic and hydrophobic amino acids played a pivotal role in antifungal activity. MtDef4 altered the permeability of fungal plasma membrane more readily than MsDef1. The antifungal activity of MsDef1, MsDef1-γ4, MtDef4, and peptides derived from the γ-core motif of each of the defensins did not rely only on their membrane permeabilizing ability. The results suggest that the γ-core motif accounts for the distinctive antifungal characteristics of each defensin (Sagaram et al. 2011).

TPP3 is a member of the class II plant defensin family from tomato. The sitting-drop vapor-diffusion method for crystal preparation and diffraction data collection to 1.7 Å resolution were employed to obtain crystals of the hexagonal space group P6(1)22, with unit-cell parameters $a = 64.97$, $b = 64.97$, $c = 82.40$ Å, $\alpha = 90$, $\beta = 90$, $\gamma = 120°$ at 293 K. (Lay et al. 2012).

7.2.5 Beta-1, 3-Glucanases

A beta-1, 3-glucanase gene (TaGluD) is a fungal defense candidate in wheat. Subsequent to *Rhizoctonia cerealis* infection, induction of its transcript in Shannong 0431, a resistant wheat line, was some 60-fold higher than that in Wenmai 6, a susceptible wheat line. The TaGluD protein was overexpressed as inclusion bodies in *Escherichia coli.* Following refolding and purification, TaGluD exerted an antifungal activity in vitro against *Alternaria longipes, Phytophthora capsici, R. cerealis,* and *R. solani,* with 30, 32, 43, and 42 % inhibition, respectively. Hence, TaGluD is exploitable for augmenting fungal resistance in crops (Liu et al. 2009).

7.2.6 Beta-Lactoglobulin-Like Protein

A dimeric 67 kDa antifungal protein from passion fruit (*Passiflora edulis*) seeds, designated as passiflin, manifests N-terminal amino acid sequence similarity to beta-lactoglobulin. The protein impeded mycelial growth in *R. solani* and breast cancer (MCF-7) cell proliferation with an IC_{50} of 16 and 15 μM, respectively. Passiflin did not cross-react with antiserum to beta-lactoglobulin. Intact beta-lactoglobulin lacks antifungal and antiproliferative activities and is much smaller

in molecular size than passiflin. However, it has been reported that hydrolyzed beta-lactoglobulin shows antifungal activity. The data suggest that passiflin is distinct from beta-lactoglobulin (Lam and Ng 2009b).

7.2.7 Lectins and Lectin-Like Proteins

The lectin from *Talisia esculent*a seeds suppressed the growth of arthroconidial chitin-rich forms of *Microsporumcanis* collected from hairs of infected animals and strains in culture. The antifungal action was abolished in the presence of N-acetyl-glucosamine and D-mannose. Fluorescent microscopy revealed the presence of the FITC-labeled lectin in macroconidial and arthroconidial forms of *M. canis.* Hence it appears that there is an association between the growth-suppressive effects of the lectin on *M. canis* and interaction of the lectin with superficial carbohydrates, predominantly D-mannose and N-acetyl-glucosamine, found on the fungi (Pinheiro et al. 2009).

Dendrobium findleyanum agglutinin was a 14.5 kDa mannose-binding protein showing 94 % homology to a *D. officinale* lectin precursor. It exerted an inhibitory action on the fungi *A. alternata* and *Collectotrichum* sp. Higher intensity of the lectin band in nearly mature and mature stages, compared to very young and young stages of the orchid pseudobulb, was observed in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Sattayasai et al. 2009)

A 30 kDa monomeric acidic lectin-like protein from the leaves of the medicinal herb *Withania somnifera*, exhibiting sequence similarity to concanavalin A, inhibited major phytopathogens under in vitro conditions. The protein brought about cell wall adhesion of the hyphae as revealed by scanning electron microscopy (Ghosh 2009).

A 29.8 kDa homodimeric sialic acid-specific lectin from *Phaseolus coccineus* seeds inhibited some plant pathogenic fungi. Subsequently, the lectin-induced apoptosis in L929 cells via a caspase-dependent pathway. The lectin also exhibits sialic acid-specificity correlates with antifungal activity and cytotoxicity (Chen et al. 2009).

A dimeric 64 kDa hemagglutinin from dried seeds of *P. vulgaris* cultivar "French bean number 35" suppressed mycelial growth in *V. mali* with an IC_{50} of 10 μM. Its hemagglutinating activity was stable in the temperature range 0–50 °C and in the pH range 6–8. It inhibited HIV-1 reverse transcriptase with an IC_{50} of 2 μM. It inhibited proliferation of hepatoma HepG2 cells and breast cancer MCF-7 cells with an IC_{50} of 100 and 2 μM, respectively. It had no antiproliferative effect on normal embryonic liver WRL68 cells (Lam and Ng 2010b).

A novel 19.8 kDa mannose-specific lectin from the roots of the traditional Chinese herbal medicine *Ophioglossum pedunculosum* inhibited the fungi *F. graminearum* and *Sclerotium rolfsii*. Its antifungal activity was stable at temperatures under 50 °C and within the pH range of 4.0–8.0. Tryptophan and arginine residues were critical to its hemagglutinating activity (He et al. 2011).

7.2.8 Lipid Transfer Proteins

Lipid transfer proteins (LTPs) facilitate the transfer of lipids between membranes in vitro. Antifungal peptides, with a 9 kDa molecular mass and an N-terminal sequence demonstrating marked semblance to those of nonspecific lipid transfer proteins, were isolated from *Brassica campestris* seeds and the mung bean. The antifungal activity of Brassica and mung bean LTPs were thermostable, pH-stable, and protease-stable. In contrast, the antifungal activity of mung bean chitinase was much less stable. Although the Brassica LTP inhibited proliferation of breast cancer MCF 7 cells and hepatoma HepG2 cells with an IC_{50} of 1.6 and 5.8 μM, respectively, and the activity of HIV-1 reverse transcriptase with an IC_{50} of 4 μM, mung bean LTP and chitinase lacked these activities. Mung bean LTP, but not Brassica LTP, exhibited antibacterial activity. All three antifungal peptides were devoid of mitogenic activity toward splenocytes. The findings suggest that the two LTPs have more desirable activities than the chitinase and that there is a dissociation between the antifungal and other activities of these antifungal proteins (Lin et al. 2007b).

A 9,412 Da antifungal peptide from *B. campestris* seeds, with an N-terminal sequence homology to lipid transfer proteins, inhibited mycelial growth in *F. oxysporum* and *M. arachidicola* with an IC_{50} value of 8.3 and 4.5 μM, respectively. It demonstrated dose-dependent binding to lyso-alpha-lauroyl phosphatidylcholine (Lin et al. 2007a). An 9.4 kDa thermostable and pH-stable antifungal peptide designated as campesin, isolated from cabbage (*B. campestris*) seeds, inhibited mycelial growth in *F. oxysporum* and *M. arachidicola*, with an IC_{50} of 5.1 and 4.4 μM, respectively. It impeded proliferation of HepG2 and MCF cancer cells with an IC_{50} of 6.4 and 1.8 μM, and inhibited HIV-1 reverse transcriptase with an IC_{50} of 3.2 μM. It demonstrated lysolecithin binding activity (Lin et al. 2009).

The peptide fraction containing the lipid transfer protein from chili pepper seeds inhibited the growth of the fungi *Colletotrichum lindemuthianum, F. oxysporum,* the yeasts*, Candida tropicalis, C. albicans, Pichia membranifaciens, and S. cerevisiae* (Cruz et al. 2010).

LTPs from *Coffea canephora* seeds inhibited *C. albicans,* and also induced morphological changes such as pseudohypha formation on *C. tropicalis*, permeabilized yeast plasma membranes to SYTOX green (Zottich et al. 2011).

Capsicum annuum lipid transfer protein, which existed in three isoforms with isoelectric points of 6.0, 8.5, and 9.5, inhibited *Colletotrichum lindemuthianum* and *C. tropicalis*, bringing about morphological alterations to the cells comprising pseudohypha formation and plasma membrane permeabilization. The protein exhibits, in addition, α-amylase inhibitory activity (Diz et al. 2011).

7.2.9 Peroxidases

An antifungal peroxidase with an optimum pH and the optimum temperature at 5.5 and 30 °C, respectively, was isolated from the large lima bean (*P. limensis*) seeds. The enzyme was stable up to 55 °C. It potently suppressed mycelial growth in *F. solani, M. arachidicola,* and *P. aphanidermatum* with an IC_{50} of 76, 103, and 119 μM, respectively. The enzyme is a basic protein with a pI of 8.6 (Wang et al. 2009c).

7.2.10 Proteases

A 43 kDa cysteine protease from *Curcuma domestica* exhibited optimal activity in the temperature range 37–60 °C. Its protease activity was inhibited by iodoacetic acid. There were peptide matches to proteasome subunit alpha type 3 of *Oryza sativa* ssp. japonica (Rice). It exhibited antifungal activity at 10 μg/ml toward pathogens *Fusarium* sp. *P. aphanidermatum,* and *T. viride* (Nagarathnam et al. 2011)

A 48 kDa protease from crude latex of a *Artocarpus heterophyllus* (jackfruit) tree demonstrated an isoelectric point of 4.2. It inhibited the growths of *P. aeruginosa* ATCC 27853 and clinical isolate of *C. albicans* at a minimum inhibitory concentration of 2.2 mg/ml and a minimum microbicidal concentration of 8.8 mg/ml (Siritapetawee et al. 2012).

7.2.11 Protease Inhibitors

A 9 kDa proteinase inhibitor from ginkgo (*Ginkgo biloba*) seeds manifesting circa 40 % sequence homology with plant type-I nonspecific lipid transfer proteins noncompetitively inhibited the aspartic acid proteinase pepsin and the cysteine proteinase papain ($K_i = 10^{-5}$–10^{-4} M). Expression of its gene was detected only in seeds, but not in leaves, roots, and stems, indicating a role in seed development and/or germination. Ginkgo proteinase inhibitor manifested lipid transfer as well as lipid-binding activity, but not antifungal and antibacterial activities. Site-directed mutagenesis study using recombinant ginkgo nsLTP1 revealed that proline (Pro)-79 and pH-80 are crucial to phospholipid transfer activity and that Pro-79 and isoleucine-82 are essential for the binding activity toward cis-unsaturated fatty acids. The alpha-helical content of P79A and F80A mutants was reduced compared with that of the wild-type protein. The papain-inhibitory activity of P79A and F80A mutants was elevated twice as much as that of the wild-type protein. Pro-79 plays a critical role in both the lipid transfer and binding activities of ginkgo nsLTP1 (Sawano et al. 2008).

ApTIA, ApTIB, and ApTIC are three approximately 20 kDa Kunitz-type serine protease inhibitor isoforms from *Acacia plumosa* seeds. Each isoforms was composed of two polypeptide chains joined by a disulfide bridge, with different acidic isoelectric points. Circular dichroism studies revealed the predominance of both disordered and beta-strands in the secondary structure of pTI isoforms as expected for beta-II proteins. The proteins were characterized by pronounced pH and thermostability The isoinhibitors could prolong, up to 10 times, the in vitro blood coagulation time and inhibited the action of trypsin ($K_i = 1.8$ nM), alpha-chymotrypsin ($K_i = 10.3$ nM), and kallikrein ($K_i = 0.58$ μM). Study of he binding of ApTIA, ApTIB, and ApTIC to trypsin and alpha-chymotrypsin by surface plasmon resonance yielded dissociation constants of 0.39, 0.56, and 0.56 nM with trypsin and 7.5, 6.9, and 3.5 nM with alpha-chymotrypsin, respectively. The growth of *A. niger, Thielaviopsis paradoxa* and *Colletotrichum* sp. P10 was also inhibited by allisoforms (Lopes et al. 2009).

A 14 kDa trypsin inhibitor from the seeds of the tartary buckwheat (*Fagopyrum tataricum*) (FtTI) with potent inhibitory activity against fungal phytopathogens possessed two disulfide bonds linking Cys(8)–Cys(65) and Cys(49)–Cys(58). Its active site had an Asp(66)–Arg(67) bond. It competitively inhibited trypsin with an inhibition constant of 1.6 nM (Ruan et al. 2011).

7.2.12 Ribonucleases

A new 15.43 kDa *Lycoris radiata* pathogenesis-related (PR)-4 gene, LrPR4 was isolated with pI of 7.56. The putative LrPR4 protein shows remarkable homology to various plant PR4 type proteins and is a member of the Barwin family. Similar to other monocot PR4 s, LrPR4 protein has a conserved Barwin domain and an N-terminal signal peptide. Recombinant LrPR4 expressed in *E. coli* hydrolyzes *L. radiata* bulb RNA and exhibits antifungal activity. Thus LrPR4 may be involved in the disease resistance responses of plants against pathogens (Li et al. 2010).

7.2.13 Thaumatin-Like Proteins

An antifungal protein named CkTLP, with sequence similarity to thaumatin-like proteins and possessing an acid cleft and a hydrophobic patch, was isolated from seeds of the desert plant *Cynanchum komarovii*. It showed antifungal activity against *B. cinerea, F. oxysporum, R. solani, V. mali* and *V. dahliae*. Its transcriptional level was upregulated in response to abscisic acid, methyl jasmonate, salicylic acid, NaCl, and drought. Thus CkTLP may play an important role in response to abiotic stresses. In transgenic Arabidopsis, CkTLP was located in the extracellular space/cell wall by CkTLP::GFP fusion protein. Overexpression of

CkTLP significantly enhanced the resistance of *Arabidopsis* against *V. dahliae* development (Wang et al. 2011).

A 20.1 kDa protein from *Calotropis procera* latex, similar to osmotin- and thaumatin-like proteins, exerted an antifungal action against *F. solani* (IC_{50} = 67.0 μg/ml), *Neurospora* sp. (IC_{50} = 57.5 μg/ml), and *Colletotrichum gloeosporioides* (IC_{50} = 32.1 μg/ml). The presence of disulfide bonds stabilizing the protein is disclosed by the decline in antifungal activity after exposure to the reducing agent dithiothreitol (de Freitas et al. 2011).

7.2.14 Other Antifungal Proteins

A 4 kDa antifungal peptide from buckwheat seeds hindered mycelial growth in *F. oxysporum* and *M. arachidicola* with an IC_{50} of 35 and 40 μM, respectively. Its antifungal activity was stable between 0 and 70 °C, and between pH 1.0/2.0 and 13. It inhibited proliferation of breast cancer (MCF-7) cells, L1210 (leukemia) cells, HepG2 (hepatoma) cells, and liver embryonic WRL 68 cells with an IC_{50} of 25, 4, 33, and 37 μM, respectively. The peptide failed to elicit a mitogenic response from murine splenocytes or enhance nitric oxide production by murine macrophages. It inhibited HIV-1 reverse transcriptase with an IC_{50} of 5.5 μM (Leung and Ng 2007).

One of the chromatographic fractions of chili pepper seeds (*C. annuum*), named F3, enriched with basic proteins with a molecular mass of 6–16 kDa, suppressed the growth of a variety of yeasts including, *C. albicans, Candida guilliermondii, Candida parapsilosis, C. tropicalis, K. marxiannus, P. membranifaciens,* and *S. cerevisiae*. It inhibited glucose-stimulated acidification of the medium by *S. cerevisiae* cells and evoked morphological alterations in different yeasts, comprising cell wall disorganization, bud formation, and pseudohypha production. The RP3 and RP4 fractions derived from fraction F3 by reverse-phase HPLC chromatography inhibited the growth of *S. cerevisiae* (Ribeiro et al. 2007).

Pomegranin from fresh pomegranate peels displayed a molecular mass of 11 kDa and an N-terminal sequence similar to that of rice disease resistance NB-S-LRR-like protein. It inhibited mycelial growth in the fungi *F. oxysporum.* and *B. cinerea* with an IC_{50} of 6.1 and 2 μM, respectively (Guo et al. 2009).

A 38 kDa protein from fresh *Capparis spinosa* melon seeds, possessing an N-terminal amino acid sequence with certain resemblance to imidazole glycerol phosphate synthase, suppressed mycelial growth in the fungus *V. mali*. It slowed proliferation of breast cancer MCF-7 cells, colon cancer HT29 cells, and hepatoma HepG2 cells, with an IC_{50} of about 60, 40, and 1 μM, respectively. It inhibited HIV-1 reverse transcriptase with IC_{50} of 0.23 μM (Lam and Ng 2009a).

Shahid et al. (2008) reported the activity-guided isolation and purification of a 50 kDa antimicrobial protein with potent, broad-spectrum antimicrobial activity from *Croton tiglium* seeds.

An optimized and established cell-suspension culture of maize (*Zea mays*) was demonstrated to constitutively secrete a number of pathogenesis-related proteins comprising the antifungal protein zeamatin (P33679) with a final yield in the vicinity of 3 mg/l medium. The zeamatin produced elicited antifungal activity toward a clinical strain of *C. albicans*, with a potency analogous to that reported for zeamatin extracted from maize kernels. A 26 kDa xylanase inhibitor, a 9 kDa lipid transfer protein, and another pathogenesis-related protein PR-5 were produced concurrently (Perri et al. 2009).

A 15 kDa antifungal protein, amaryllin, from the underground bulbs of *Amaryllis belladonna,* showed antifungal activity against *Aspergillus flavus* and *F. oxysporum*. The protein was crystallized using the hanging-drop vapor-diffusion method with 30 % PEG 8000 as precipitating agent. The crystals diffracted to 2.7 A/orthorhombic space group I222 or I2(1)2(1)2(1), with unit-cell parameters $a = 48.6$, $b = 61.9$, $c = 79.6$ A (Kumar et al. 2009).

A 5,907 Da antifungal peptide from kale seeds with striking pH stability and thermostability inhibited mycelial growth in various fungi comprising *F. oxysporum, H. maydis, M. arachidicola* and *V. mali*. The IC_{50} values were, respectively, 4.3, 2.1, 2.4, and 0.15 μM. It retarded proliferation of breast cancer (MCF7) cells and hepatoma (HepG2) with an IC_{50} of 3.4 and 2.7 μM, and inhibited the activity of HIV-1 reverse transcriptase with an IC_{50} of 4.9 μM (Lin and Ng 2008).

A 5,716 Da antifungal peptide from *Brassica parachinensis* seeds, designated as brassiparin, potently curtailed mycelial growth in the fungi *F. oxysporum, H. maydis, M. arachidicola* and *V. mali.* The antifungal activity toward *M. arachidicola* was exceedingly thermostable and pH-stable. It inhibited proliferation of breast cancer (MCF7) and hepatoma (HepG2) cells and the activity of HIV-1 reverse transcriptase (Lin and Ng 2009).

The 18.9 kDa juncin from Japanese takana (*Brassica juncea* var. integrifolia) seeds inhibited mycelial growth in the phytopathogenic fungi *F. oxysporum, M. arachidicola,* and *H. maydis,* with IC_{50} values of 3.5, 10, and 27 μM, respectively. Proliferation of breast cancer (MCF7) and hepatoma (HepG2) cells and the activity of HIV-1 reverse transcriptase were inhibited with IC_{50} values of 6.4, 5.6, and 4.5 μM, respectively It was devoid of mitogenic activity toward splenocytes and nitric oxide inducing activity toward macrophages. Juncin differed in N-terminal sequence differed in molecular mass and N-terminal amino acid sequence from *Brassica campestris* and *Brassica alboglabra* antifungal peptides, but displayed similar biological activities (Ye and Ng 2009).

A 30 kDa antifungal protein from red cabbage (*Brassica oleracea*) seeds retarded mycelial growth in *Bipolaris maydis, M. arachidicola*, *Setospaeria turcica,* and *C. albicans*. The antifungal activity remained intact from pH 3 to 11 and from 0 to 65 °C. The antifungal action was achieved by permeabilizing the fungal membrane as witnessed by Sytox green staining. The antifungal protein exerted an antibacterial action against *P. aeruginosa* ($IC_{50} = 53$ μM). Proliferation of nasopharyngeal cancer and hepatoma cells was suppressed with IC_{50} of 50 and 90 μM, respectively, after 48 h of culture in the presence of the antifungal protein (Ye et al. 2011)

Canola (*Brassica napus*), an agriculturally important oilseed crop, can be adversely affected by diseases such as alternaria black spot, blackleg, and sclerotinia stem rot, ensuing in a considerable deterioration of crop productivity and quality. The cDNA encoding PmAMP1, an antimicrobial peptide isolated from western white pine (*Pinus monticola*), has been successfully incorporated into the genome of *B. napus.* In planta expression of PmAMP1 conferred protection against *Alternaria brassicae, Leptosphaeria maculans* and *Sclerotinia sclerotiorum* (Verma et al. 2012)

A 14,865 Da thermostable antifungal protein (Pr-2) void of cytotoxicity was identified from pumpkin rinds. The protein potently inhibited, at 10–20 μM, the growth of *B. cinerea, C. coccodes, F. oxysporum, F. solani,* and *Trichoderma harzianum* in vitro. It targets the fungal cell membrane (Park et al. 2009).

Acafusin from *A. confusa* seeds was a dimeric 34 kDa antifungal protein. It inhibited mycelial growth in *R. solani* and HIV-1 reverse transcriptase with an IC_{50} of 28 and 80 μM, respectively (Lam and Ng 2011b).

The 14 kDa protein from the *Aloe vera* leaf gel exhibited a potent anti-fungal activity against *C. albicans, Candida krusei,* and *C. parapsilosis.* It demonstrated an anti-inflammatory activity as judged by 84 and 73 % inhibition of lipoxygenase and cyclooxygenase-2 activity (Das et al. 2011).

Two antifungal proteins from rosemary pepper (*Lippia sidoides*) flowers with a molecular mass of 10 and 15 kDa, respectively, inhibited *B. cinerea*, but did not inhibit all bacterial species examined. Both proteins showed N-terminal sequence homology with NBS-LRRR proteins (Moreira et al. 2011).

A 26.9 kDa antifungal peptide from dry seeds of the foxtail millet (*Setaria italica*.) inhibited mycelial growth in *A. alternate* with an IC_{50} of 1.3 μM, and also in *B. cinerea, F. oxysporum*, and *T. viride*. Drastic ultrastructural changes in the mold forms of *A. alternate* were revealed following exposure to 20 μg/ml of the antifungal protein for 48 h (Xu et al. 2011).

A 7.3 kDa antifungal peptide from dried red kidney beans slowed mycelial growth in *F. oxysporum* with an IC_{50} of 3.8 ± 0.4 μM and also in *M. arachidicola*. It hampered growth of leukemia L1210 cells and lymphoma MBL2cells with an IC_{50} of 7.6 ± 0.6 and 5.2 ± 0.4 μM, respectively. HIV-1 reverse transcriptase was inhibited with an IC_{50} of 40 ± 3.2 μM. However, no inhibitory activity on other viral enzymes was observed (Li et al. 2011).

A 50 kDa antifungal protein from *Sesbania virgata* seeds inhibited the filamentous fungi *A. niger, Cladosporium cladosporioides, C. gloeosporioides* and *F. solani* (Praxedes et al. 2011).

7.3 Conclusion

From the foregoing account, an impression can be gathered that plants elaborate a spectacular array of antifungal proteins and peptides. Although they share the defense function of combating pathogenic fungi which can inflict massive damage,

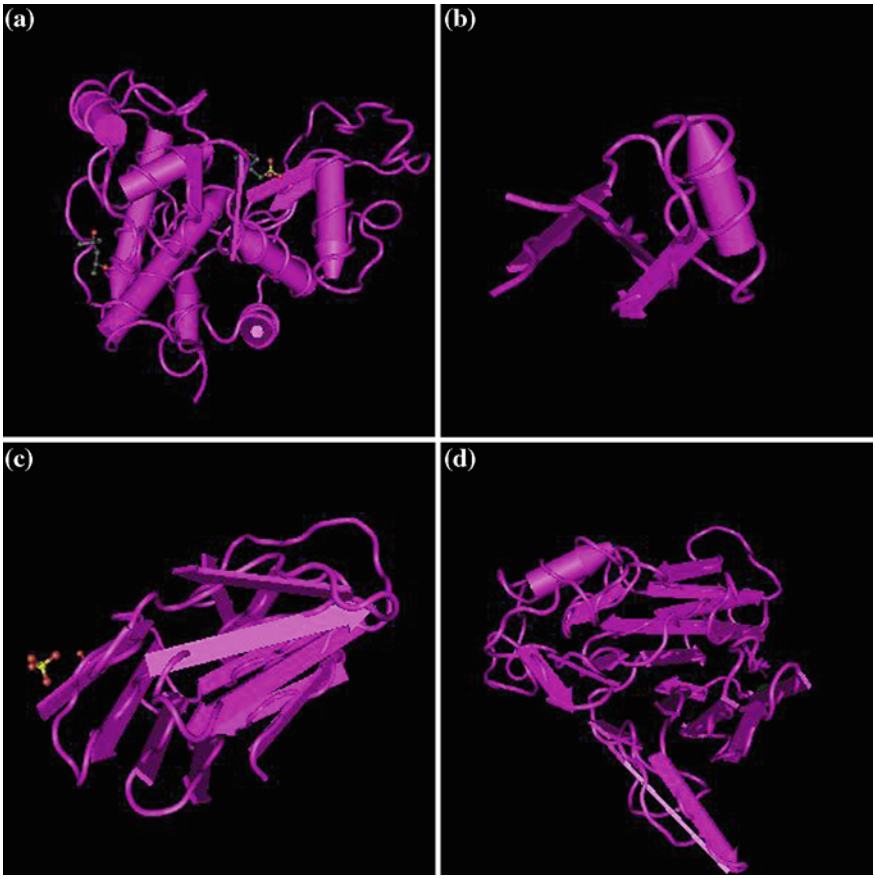

Fig. 7.1 Structure of different classes of proteins with antifungal activities. **a** Class I Chitinase from *Oryza Sativ*a L. Japonica (Mizuno et al. 2008). **b** Defensin from *Pisum sativum* (Almeida et al. 2002). **c** Lectins from *Gastrodia elata* (Liu et al. 2005). **d** Thaumatin-like protein from *Solanum lycopersicum* (Ghosh and Chakrabarti 2008)

they display distinctly different structures (Fig. 7.1 and Table 7.1). Some of these antifungal proteins manifest desirable characteristics including a broad-spectrum antifungal action, lack of toxicity, a small molecular size, and outstanding thermostability and pH stability. In case of antifungal proteins and peptides such as defensins, attempts have been made to unravel the antifungal mechanism. Permeabilization of fungal membranes, induction of reactive oxygen species, and triggering of apoptotic changes are implicated. The chitinase and glucanase activities of some antifungal proteins may contribute to their antifungal activity. It is noteworthy that plant antifungal proteins and peptides are efficacious against phytopathogens as well as fungi pathogenic to humans, e.g. *C. albicans* and related species. Due to the need for new antifungals to circumvent the development of fungal resistance to existing therapeutics, these plant antifungal proteins and

Table 7.1 N-terminal sequences of some plant antifungal proteins

2S albumin (*Taraxacum officinale*) Odintsova et al. 2010	PVSRQQCSQR	IQGERFNQCR	SQMQDGQLQS
Amidase (*Peltophorum pterocarpum*) Lam and Ng 2010a	FEFKEATIDG	IQNF	
Chitinase (*Bambusa oldhamii*) Kuo et al. 2008	MVAKLKWSPL	LPLLLLLAGM	VGMSRAGNIA
Chitinase (*Acacia confusa*) Lam and Ng 2011a	EQHGRQAGGA	LCMGG	
Defensin (Phaseolus vulgaris cv. 'French bean') Leung et al. 2008	KTCENLADTY		
Defensin (Phaseolus vulgaris cv. 'Cloud bean') Wu et al. 2011	KTYENLADTY	KGPYFTTGSH	DDHYKNKEHL
Defensin (*Phaseolus angularis* cv. 'Nepalese large red beans') Ma et al. 2009	KTYENLADTY	KGPYFTTGSH	DDHYKNKEHL
Defensin (*Phaseolus limensis*) Wang et al. 2009a	KTCENLATYY	YRGPCF	
Beta-lactoglobulin-like peptide (*Passiflora edulis*) Lam and Ng 2009b	AFLDIQKVAG	TWYSLA	
Lectin (*Dendrobium findlayanum*) Sattayasai et al. 2009	MAFSISSTMI	FLLSLALFST	LVSADNHLLP
Lectin (*Phaseolus coccineus*) Chen et al. 2009	ATETSFSFQR	LNLANLVLNK	ESS
Lectin (Phaseolus vulgaris cv. 'French Bean No. 35') Lam and Ng 2010b	ATETYSAFQR	FCETNLILQR	
Lipid transfer protein (*Brassica campestris* L. var. purpurea Bailey) Lin et al. 2007a	ALSCGTVSGN	LAACAGYV	
Lipid transfer protein (*Brassica campestris*) Lin et al. 2009	ALSCGTVSAQ	IAACAGYV	
Protease Inhibitor (*Fagopyrum tataricum*) Ruan et al. 2011	LIYAKVECLT	TGVRTYVGKQ	SWPELVGTKG
Protease Inhibitor (*Ginkgo biloba*) Sawano et al. 2008	MMKISWQLWL	LVAFAVMVCV	WTPLSTAAPG
Thaumatin-like protein (*Calotropis procera*) de Freitas et al. 2011	ATFTIRNNCP	YTIWAAAVPG	GRRLNSGGT
Ribonuclease (*Lycoris radiata*) Li et al. 2010	MAMERVSLVI	VLLLGLAAAS	FAQQASNVRA
Other antifungal protein (*Fagopyrum esculentum*) Leung and Ng 2007	AQCGAQGGGA	TCPGG	
Other antifungal protein (*Capparis spinosa*) Lam and Ng 2009a	SYDTQAEAAL		
Other antifungal protein (*Amaryllis belladonna*) Kumar et al. 2009	QKIQEIDLQT	YLQPO	
Other antifungal protein (*Brassica alboglabra*) Lin and Ng 2008	PEGPFQGPKA	TKPGDLAXQT	WGGWXGQTPK
Other antifungal protein (*Brassica parachinensis*) Lin and Ng 2009	DQFPQEYPGD	VQFSFN	
Other antifungal protein (*Brassica juncea* var. integrifolia) Ye and Ng 2009	GVEVTRELRS	ERPSGKIVTI	
Other antifungal protein (*Brassica oleracea*) Ye et al. 2011	RQGPTTGPTR	KATGL	
Other antifungal protein (*Cucurbita moschata*) Park et al. 2009	QGIGVGDNDG	KRGKR	
Other antifungal protein (*Lippia sidoides*) Moreira et al. 2011	EALYNSEDLY	EETSDSDD	
	SPPEAAYGPG	NTNSDSQDK	
Other antifungal protein (Red kidney bean) Li et al. 2011	DGVCFGGLAN	GDRT	
Other antifungal protein (*Sesbania virgata*) Praxedes et al. 2011	AMVHSPGGFS		

peptides may be exploitable as an alternative solution. It is well documented that transgenic plants overexpressing antifungal proteins and peptides acquire an augmented ability to resist fungal pathogens. Undoubtedly, basic and applied research on antifungal proteins and peptides will continue to contribute to the welfare of mankind.

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References

Aerts AM, François IE, Meert EM, Li QT, Cammue BP, Thevissen K (2007) The antifungal activity of RsAFP2, a plant defensin from *Raphanus sativus*, involves the induction of reactive oxygen species in *Candida albicans*. J Mol Microbiol Biotechnol 13:243–247

Almeida MS, Cabral KM, Kurtenbach E, Almeida FC, Valente AP (2002) Solution structure of Pisum sativum defensin 1 by high resolution NMR: plant defensins, identical backbone with different mechanisms of action. J Mol Biol 315:749–757

Ceasar SA, Ignacimuthu S (2012) Genetic engineering of crop plants for fungal resistance: role of antifungal genes. Biotechnol Lett 34:995–1002

Chen J, Liu B, Ji N, Zhou J, Bian HJ, Li CY, Chen F, Bao JK (2009) A novel sialic acid-specific lectin from *Phaseolus coccineus* seeds with potent antineoplastic and antifungal activities. Phytomedicine 16:352–360

Cruz LP, Ribeiro SF, Carvalho AO, Vasconcelos IM, Rodrigues R, Da Cunha M, Gomes VM (2010) Isolation and partial characterization of a novel lipid transfer protein (LTP) and antifungal activity of peptides from chilli pepper seeds. Protein Pept Lett 17:311–318

Das S, Mishra B, Gill K, Ashraf MS, Singh AK, Sinha M, Sharma S, Xess I, Dalal K, Singh TP, Dey S (2011) Isolation and characterization of novel protein with anti-fungal and anti-inflammatory properties from *Aloe vera* leaf gel. Int J Biol Macromol 48:38–43

de Beer A, Vivier MA (2008) Vv-AMP1, a ripening induced peptide from *Vitis vinifera* shows strong antifungal activity. BMC Plant Biol 8:75

de Freitas CD, Nogueira FC, Vasconcelos IM, Oliveira JT, Domont GB, Ramos MV (2011) Osmotin purified from the latex of *Calotropis procera*: biochemical characterization, biological activity and role in plant defense. Plant Physiol Biochem 49:738–743

De-Paula VS, Razzera G, Medeiros L, Miyamoto CA, Almeida MS, Kurtenbach E, Almeida FC, Valente AP (2008) Evolutionary relationship between defensins in the Poaceae family strengthened by the characterization of new sugarcane defensins. Plant Mol Biol 68:321–335

Diz MS, Carvalho AO, Ribeiro SF, Da Cunha M, Beltramini L, Rodrigues R, Nascimento VV, Machado OL, Gomes VM (2011) Characterisation, immunolocalisation and antifungal activity of a lipid transfer protein from chili pepper (*Capsicum annuum*) seeds with novel α-amylase inhibitory properties. Physiol Plant 142:233–246

Games PD, Dos Santos IS, Mello EO, Diz MS, Carvalho AO, de Souza-Filho GA, Da Cunha M, Vasconcelos IM, Ferreira Bdos S, Gomes VM (2008) Isolation, characterization and cloning of a cDNA encoding a new antifungal defensin from *Phaseolus vulgaris* L. seeds. Peptides 29:2090–2100

Ghosh M (2009) Purification of a lectin-like antifungal protein from the medicinal herb, *Withania somnifera*. Fitoterapia 80:91–95

Ghosh R, Chakrabarti C (2008) Crystal structure analysis of NP24-I: a thaumatin-like protein. Planta 228:883–890

Guo G, Wang HX, Ng TB (2009) Pomegranin, an antifungal peptide from pomegranate peels. Protein Pept Lett 16:82–85

He XM, Ji N, Xiang XC, Luo P, Bao JK (2011) Purification, characterization, and molecular cloning of a novel antifungal lectin from the roots of *Ophioglossum pedunculosum*. Appl Biochem Biotechnol 165:1458–1472

Ho VS, Ng TB (2007) Chitinase-like proteins with antifungal activity from emperor banana fruits. Protein Pept Lett 14:828–831

Kant P, Liu WZ, Pauls KP (2009) PDC1, a corn defensin peptide expressed in *Escherichia coli* and *Pichia pastoris* inhibits growth of *Fusarium graminearum*. Peptides 30:1593–1599

Kim JY, Park SC, Hwang I, Cheong H, Nah JW, Hahm KS, Park Y (2009) Protease inhibitors from plants with antimicrobial activity. Int J Mol Sci 10:2860–2872

Kovaleva V, Krynytskyy H, Gout I, Gout R (2011) Recombinant expression, affinity purification and functional characterization of Scots pine defensin 1. Appl Microbiol Biotechnol 89:1093–1101

Kovalskaya N, Hammond RW (2009) Expression and functional characterization of the plant antimicrobial snakin-1 and defensin recombinant proteins. Protein Expr Purif 63:12–17

Kumar S, Singh N, Sinha M, Kaur P, Srinivasan A, Sharma S, Singh TP (2009) Isolation, purification, crystallization and preliminary crystallographic studies of amaryllin, a plant pathogenesis-related protein from *Amaryllis belladonna*. Acta Crystallogr Sect F Struct Biol Cryst Commun 65:635–637

Kuo CJ, Liao YC, Yang JH, Huang LC, Chang CT, Sung HY (2008) Cloning and characterization of an antifungal class III chitinase from suspension-cultured bamboo (*Bambusa oldhamii*) cells. J Agric Food Chem 56:11507–11514

Lam SK, Ng TB (2009a) A protein with antiproliferative, antifungal and HIV-1 reverse transcriptase inhibitory activities from caper (*Capparis spinosa*) seeds. Phytomedicine 16:444–450

Lam SK, Ng TB (2009b) Passiflin, a novel dimeric antifungal protein from seeds of the passion fruit. Phytomedicine 16:172–180

Lam SK, Ng TB (2010a) First report of an antifungal amidase from Peltophorum pterocarpum. Biomed Chromatogr 24:458–464 (Erratum in: Biomed Chromatogr 24:798)

Lam SK, Ng TB (2010b) Isolation and characterization of a French bean hemagglutinin with antitumor, antifungal, and anti-HIV-1 reverse transcriptase activities and an exceptionally high yield. Phytomedicine 17:457–462

Lam SK, Ng TB (2011a) Acaconin, a chitinase-like antifungal protein with cytotoxic and anti-HIV-1 reverse transcriptase activities from *Acacia confusa* seeds. Acta Biochim Pol 7:299–304

Lam SK, Ng TB (2011b) Acafusin, a dimeric antifungal protein from *Acacia confus*a seeds. Protein Pept Lett 17:817–822

Lay FT, Veneer PK, Hulett MD, Kvansakul M (2012) Recombinant expression and purification of the tomato defensin TPP3 and its preliminary X-ray crystallographic analysis. Acta Crystallogr Sect F Struct Biol Cryst Commun 1:314–316

Leung EH, Ng TB (2007) A relatively stable antifungal peptide from buckwheat seeds with antiproliferative activity toward cancer cells. J Pept Sci 13:762–767

Leung EH, Wong JH, Ng TB (2008) Concurrent purification of two defense proteins from French bean seeds: a defensin-like antifungal peptide and a hemagglutinin. J Pept Sci 14:349–353

Li X, Xia B, Jiang Y, Wu Q, Wang C, He L, Peng F, Wang R (2010) A new pathogenesis-related protein, LrPR4, from *Lycoris radiata*, and its antifungal activity against *Magnaporthe grisea*. Mol Biol Rep 37:995–1001

Li M, Wang H, Ng TB (2011) An antifungal peptide with antiproliferative activity toward tumor cells from red kidney beans. Protein Pept Lett 18:594–600

Lin P, Ng TB (2008) A novel and exploitable antifungal peptide from kale (*Brassica alboglabra*) seeds. Peptides 29:1664–1671

Lin P, Ng TB (2009) Brassiparin, an antifungal peptide from *Brassica parachinensis* seeds. J Appl Microbiol 106:554–563

Lin P, Xia L, Ng TB (2007a) First isolation of an antifungal lipid transfer peptide from seeds of a Brassica species. Peptides 28:1514–1519

Lin P, Xia L, Wong JH, Ng TB, Ye X, Wang S, Shi X (2007b) Lipid transfer proteins from *Brassica campestris* and mung bean surpass mung bean chitinase in exploitability. J Pept Sci 13:642–648

Lin P, Wong JH, Xia L, Ng TB (2009) Campesin, a thermostable antifungal peptide with highly potent antipathogenic activities. J Biosci Bioeng 108:259–265

Liu W, Yang N, Ding J, Huang RH, Hu Z, Wang DC (2005) Structural mechanism governing the quaternary organization of monocot mannose-binding lectin revealed by the novel monomeric structure of an orchid lectin. J Biol Chem 280:14865–14876

Liu B, Lu Y, Xin Z, Zhang Z (2009) Identification and antifungal assay of a wheat beta-1, 3-glucanase. Biotechnol Lett 31:1005–1010

Lopes JL, Valadares NF, Moraes DI, Rosa JC, Araújo HS, Beltramini LM (2009) Physicochemical and antifungal properties of protease inhibitors from *Acacia plumose*. Phytochemistry 70:871–879

Ma DZ, Wang HX, Ng TB (2009) A peptide with potent antifungal and antiproliferative activities from Nepalese large red beans. Peptides 30:2089–2094

Menegassi A, Wassermann GE, Olivera-Severo D, Becker-Ritt AB, Martinelli AH, Feder V, Carlini CR (2008) Urease from cotton (*Gossypium hirsutum*) seeds: isolation, physicochemical characterization, and antifungal properties of the protein. J Agric Food Chem 56:4399–4405

Moreira JS, Almeida RG, Tavares LS, Santos MO, Viccini LF, Vasconcelos IM, Oliveira JT, Raposo NR, Dias SC, Franco OL (2011) Identification of botryticidal proteins with similarity to NBS-LRR proteins in rosemary pepper (*Lippia sidoides* Cham.) flowers. Protein J 30:32–38

Nagarathnam R, Rengasamy A, Balasubramanian R (2011) Purification and properties of cysteine protease from rhizomes of Curcuma longa (Linn.). J Sci Food Agric 90:97–105 (Erratum in: J Sci Food Agric 90:541)

Odintsova TI, Rogozhin EA, Sklyar IV, Musolyamov AK, Kudryavtsev AM, Pukhalsky VA, Smirnov AN, Grishin EV, Egorov TA (2010) Antifungal activity of storage 2S albumins from seeds of the invasive weed dandelion *Taraxacum officinale* Wigg. Protein Pept Lett 17:522–529

Park SC, Kim JY, Lee JK, Hwang I, Cheong H, Nah JW, Hahm KS, Park Y (2009) Antifungal mechanism of a novel antifungal protein from pumpkin rinds against various fungal pathogens. J Agric Food Chem 57:9299–9304

Perri F, Della Penna S, Rufini F, Patamia M, Bonito M, Angiolella L, Vitali A (2009) Antifungal-protein production in maize (*Zea mays*) suspension cultures. Biotechnol Appl Biochem 52:273–281

Pinheiro AQ, Melo DF, Macedo LM, Freire MG, Rocha MF, Sidrim JJ, Brilhante RS, Teixeira EH, Campello CC, Pinheiro DC, Lima MG (2009) Antifungal and marker effects of *Talisia esculenta* lectin on *Microsporum canis* in vitro. J Appl Microbiol 107: 2063–2069

Praxedes PG, Zerlin JK, Dias LO, Pessoni RA (2011) A novel antifungal protein from seeds of Sesbania virgata (Cav.) Pers. (*Leguminosae-Faboideae*). Braz J Biol 71:687–692

Ribeiro SF, Carvalho AO, Da Cunha M, Rodrigues R, Cruz LP, Melo VM, Vasconcelos IM, Melo EJ, Gomes VM (2007) Isolation and characterization of novel peptides from chilli pepper seeds: antimicrobial activities against pathogenic yeasts. Toxicon 50:600–611

Rivillas-Acevedo LA, Soriano-García M (2007) Isolation and biochemical characterization of an antifungal peptide from *Amaranthus hypochondriacus* seeds. J Agric Food Chem 55:10156–10161

Ruan JJ, Chen H, Shao JR, Wu Q, Han XY (2011) An antifungal peptide from *Fagopyrum tataricum* seeds. Peptides 32:1151–1158

Sagaram US, Pandurangi R, Kaur J, Smith TJ, Shah DM (2011) Structure-activity determinants in antifungal plant defensins MsDef1 and MtDef4 with different modes of action against *Fusarium graminearum*. PLoS One 6:e18550

Sattayasai N, Sudmoon R, Nuchadomrong S, Chaveerach A, Kuehnle AR, Mudalige-Jayawickrama RG, Bunyatratchata W (2009) *Dendrobium findleyanum* agglutinin: production, localization, anti-fungal activity and gene characterization. Plant Cell Rep 28:1243–1252

Sawano Y, Hatano K, Miyakawa T, Komagata H, Miyauchi Y, Yamazaki H, Tanokura M (2008) Proteinase inhibitor from ginkgo seeds is a member of the plant nonspecific lipid transfer protein gene family. Plant Physiol 146:1909–1919

Shahid M, Tayyab M, Naz F, Jamil A, Ashraf M, Gilani AH (2008) Activity-guided isolation of a novel protein from *Croton tiglium* with antifungal and antibacterial activities. Phytother Res 22:1646–1649

Siritapetawee J, Thammasirirak S, Samosornsuk W (2012) Antimicrobial activity of a 48 kDa protease (AMP48) from *Artocarpus heterophyllus* latex. Eur Rev Med Pharmacol Sci 16:132–137

Slavokhotova AA, Odintsova TI, Rogozhin EA, Musolyamov AK, Andreev YA, Grishin EV, Egorov TA (2011) Isolation, molecular cloning and antimicrobial activity of novel defensins from common chickweed (*Stellaria media* L.) seeds. Biochimie 93:450–456

Tavares PM, Thevissen K, Cammue BP, François IE, Barreto-Bergter E, Taborda CP, Marques AF, Rodrigues ML, Nimrichter L (2008) *In vitro* activity of the antifungal plant defensin RsAFP2 against Candida isolates and its in vivo efficacy in prophylactic murine models of candidiasis. Antimicrob Agents Chemother 52:4522–4525

Verma SS, Yajima WR, Rahman MH, Shah S, Liu JJ, Ekramoddoullah AK, Kav NN (2012) A cysteine-rich antimicrobial peptide from *Pinus monticola* (PmAMP1) confers resistance to multiple fungal pathogens in canola (*Brassica napus*). Plant Mol Biol 79:61–74

Wang S, Shao B, Rao P, Lee Y, Ye X (2007) Hypotin, a novel antipathogenic and antiproliferative protein from peanuts with a sequence similar to those of chitinase precursors. J Agric Food Chem 55:9792–9799

Wang S, Rao P, Ye X (2009a) Isolation and biochemical characterization of a novel leguminous defense peptide with antifungal and antiproliferative potency. Appl Microbiol Biotechnol 82:79–86

Wang S, Shao B, Fu H, Rao P (2009b) Isolation of a thermostable legume chitinase and study on the antifungal activity. Appl Microbiol Biotechnol 85:313–321

Wang SY, Gong YS, Zhou JJ (2009c) Chromatographic isolation and characterization of a novel peroxidase from large lima legumes. J Food Sci 74:C193–C198

Wang Q, Li F, Zhang X, Zhang Y, Hou Y, Zhang S, Wu Z (2011) Purification and characterization of a CkTLP protein from *Cynanchum komarovii* seeds that confers antifungal activity. PLoS One 6:e16930

Wu X, Sun J, Zhang G, Wang H, Ng TB (2011) An antifungal defensin from *Phaseolus vulgaris* cv. 'Cloud Bean'. Phytomedicine 18:104–109

Xu W, Wei L, Qu W, Liang Z, Wang J, Peng X, Zhang Y, Huang K (2011) A novel antifungal peptide from foxtail millet seeds. J Sci Food Agric 91:1630–1637

Yan R, Hou J, Ding D, Guan W, Wang C, Wu Z, Li M (2008) *In vitro* antifungal activity and mechanism of action of chitinase against four plant pathogenic fungi. J Basic Microbiol 48:293–301

Ye X, Ng TB (2009) Isolation and characterization of juncin, an antifungal protein from seeds of Japanese Takana (*Brassica juncea* Var. integrifolia). J Agric Food Chem 57:4366–4371

Ye XJ, Ng TB, Wu ZJ, Xie LH, Fang EF, Wong JH, Pan WL, Wing SS, Zhang YB (2011) Protein from red cabbage (*Brassica oleracea*) seeds with antifungal, antibacterial, and anticancer activities. J Agric Food Chem 59:10232–10238

Zhao M, Ma Y, Pan YH, Zhang CH, Yuan WX (2011) A hevein-like protein and a class I chitinase with antifungal activity from leaves of the paper mulberry. Biomed Chromatogr 25:908–912

Zottich U, Da Cunha M, Carvalho AO, Dias GB, Silva NC, Santos IS, do Nacimento VV, Miguel EC, Machado OL, Gomes VM (2011) Purification, biochemical characterization and antifungal activity of a new lipid transfer protein (LTP) from *C. canephora* seeds with α-amylase inhibitor properties. Biochim Biophys Acta 1810:375–383

Chapter 8
Antifungal Metabolites of Endophytic Fungi

Karsten Krohn and Barbara Schulz

Abstract Not only medicinal plants, but also the endophytic fungi that colonize them are excellent sources of antifungal metabolites. In some cases, the substances detected in plants have been shown to be secondary metabolites synthesized by their fungal inhabitants. As we have shown, most endophytic fungi produce biologically active metabolites, many of which are antifungal. We speculate that in situ these antifungal metabolites protect the endophytic fungi from competitors within the plant host. The metabolites belong to diverse structural groups and include many unprecedented carbon skeletons, but also derivatives of known structural groups: the benzofuranes, biaryl ethers, botryanes, coniothyriomycins, dinemasones, epoxydon derivatives, fusidilactones, isocoumarins, massarilactones, palmarumycins, pestalotheols, pyranocines, pyrenocines, naphthalenes, oblongolides and xanthones. The chemical diversity of the fungal metabolites by functional group transformation and the quality of diversity by ring closure of open-chain compounds and dimerization is demonstrated with examples from our research. We conclude that endophytic fungi are creative masters of chemical synthesis.

8.1 Introduction

Medicinal Plants continue to be a valuable source of pharmaceutically important compounds (Butler 2004; Baker et al. 2007; Newman and Cragg 2007). However, in many cases, the active compounds isolated from the plant may have been

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produced by the endophytic fungi that colonize it (Zhang et al. 2006). Thus, a report on antifungal metabolites could compliment the other contributions to this book that report on antifungal compounds present in medicinal plants. In fact, in our almost 30 years of research, antifungal metabolites were detected in the culture extracts of most of the investigated endophytes. A reason for this could be the protection against competing fungi in the same host plant. The role of microorganisms in the discovery and development of pharmaceutical products was described in a recent review (Pearce et al. 2010); the specific potential of fungi in the discovery of novel low-molecular weight pharmaceuticals was discussed in a further publication (Dreyfuss and Chapela 1994). General information on screening, cultivation (Schulz et al. 2008) and structure elucidation can be found in several reviews (Schulz et al. 1995, 2002; Krohn and Schulz 2011).

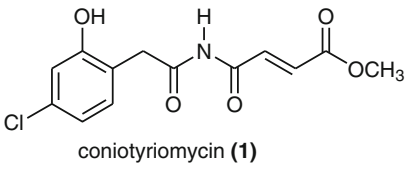
In this chapter, we describe the antifungal compounds produced by endophytic fungi, arranged by chemical compound classes.

The first structurally comparatively easy, but highly antifungal metabolite, isolated from an unidentified *Coniothyrium* sp. was an open chain imide, named coniothyriomycin (**1**) (Fig. 8.1) (Krohn et al. 1992). This metabolite is included here, although the fungus was isolated from a soil sample from Mali. It belongs to the genus, *Coniothyrium,* of the Fungi imperfecti.

The compound is decomposed in protic solvents such as methanol or water by hydrolysis of the imide functionality. Therefore, in spite of very good short time antifungal activities in vitro as well as in vivo, the compound had no long-term or curative activity.

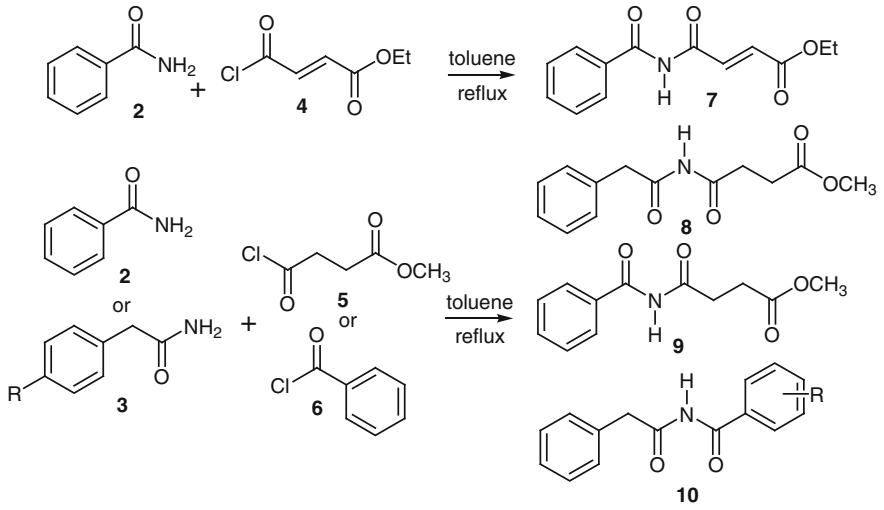
To improve the stability and hopefully the antifungal activities, an extensive synthetic study of the analogs was undertaken. The structure of the antifungal metabolite coniothyriomycin was systematically modified by changing the acids of the open chain imide, modification of the hydrophobicity, variation in the degree of saturation, replacement of carbons by nitrogen or oxygen, and incorporation of the open chain molecule into cyclic arrangements (Krohn et al. 2003). Thus, as shown in Scheme 8.1, benzamide (**2**) or phenylacetic acid (**3**) was reacted with the monochlorides (**4**) of malonic or succinic acid monomethyl ester chloride (**5** or **6**) to afford the condensed imides **7**–**10**.

In another series, it was possible to introduce an additional nitrogen or oxygen atom into the molecule by condensation of the malic ester monochloride (**13**) with the hydrazide **11** or the benzoylbenzamide (**12**) to afford the analogs **14** or **15**.

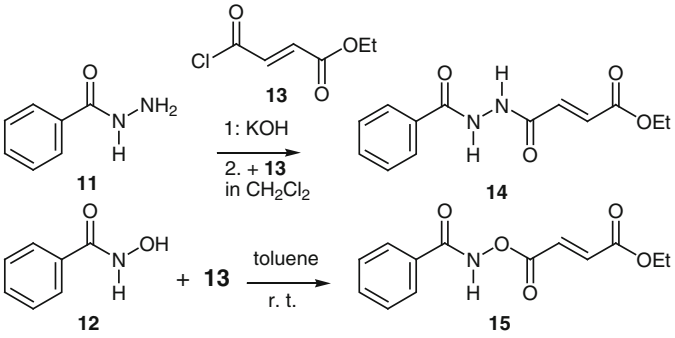


coniotyriomycin **(1)**

Fig. 8.1 Structure of coniothyriomycin (**1**)



Scheme 8.1 Synthesis of coniothyriomycins by changing the chain length of the benzamide and diacid components (Krohn et al. 2003)



Structure–activity studies showed that antifungal activity was retained by replacement of phenylacetic acids by benzoic acids in the imide structure such as **7**, but diminished by hydrogenation of the fumaric ester part as in compounds **8**–**10** (Krohn et al. 2003).

The tested synthetic coniothyriomycin analogs primarily showed control of plant diseases caused by representatives of the fungal-like Oomycetes, e.g., late blight on tomatoes caused by *Phytophthora infestans* or downy mildew on grape vine caused by *Plasmopara viticola*. The inhibitions were found both in vitro and on intact plants in a greenhouse.

Changing the molecular fragment from phenylacetic amide to benzoic amide in **1**–**5 h** retained the same good in vitro activity as the lead structure coniothyriomycin had had. Compounds **5a** to **7** controlled *P. infestans*. Also, control of the causal organism of rice blast, *Pyricularia oryzae,* and of the causal organism of leaf blotch on wheat, *Septoria tritici,* was observed. The hydrogenation of the

double bond in the fumaric acid fragment, which resulted in **8** and **9,** led to total loss of fungicidal activity both in vitro and in vivo. The other structural variations in the compounds resulted in reduced in vitro activity against *P. infestans.*

The naphthol (**16**) and the new nitronaphthalenes **17**–**20** and ergosterol (**21**) (Fig. 8.2), usually present in fungal cultures, were isolated from another *Coniothyrium* species, an endophyte in the shrub *Sideritis chamaedryfolia*, from an arid habitat near Alicante, Spain (Krohn et al. 2008a). The culture extract of the fungus was found to have strong antibacterial, antifungal, antialgal, and herbicidal activities. The nitronaphthalenes **17**–**20** were known from chemical synthesis, but new as natural products. Compounds **17**–**19** showed very good antifungal activity against *Microbotryum violaceum.* Compound **19** was also active against the bacteria *Bacillus megaterium* and *Escherichia coli* and the algae *Chlorella fusca* (Krohn et al. 2008a).

Likewise, derivatives of naphthalenes **22**–**24** were isolated as dimers of 8-methoxy-naphthol from the endophytic fungus *Nodulisporium* sp. from *Juniperus cedre* from Gomera (Dai et al. 2009). The dimeric naphthalenes nodulisporin A (**23**) and B (**24**) were new natural products, whereas daldinol (**22**) was known. The new dimers **23** and **24** showed antifungal activity against *M. violaceum* (Dai et al. 2009) (Fig. 8.3).

The dimeric naphthalenes **22** and **24** were also prepared synthetically by oxidative dimerization using ammonium metapervanadate (NH_4VO_3) (Krohn and Aslan 2009).

Structurally related metabolites, this time produced by coupling of the 8-methoxy-naphthol to a benzopyran unit, were isolated from the culture of another endophytic *Nodulisporium* sp., which had been isolated from *Erica arborea,* also from Gomera (Fig. 8.4) (Dai et al. 2009). Interestingly, all of the monomers were also found in both fungi that produced the dimer.

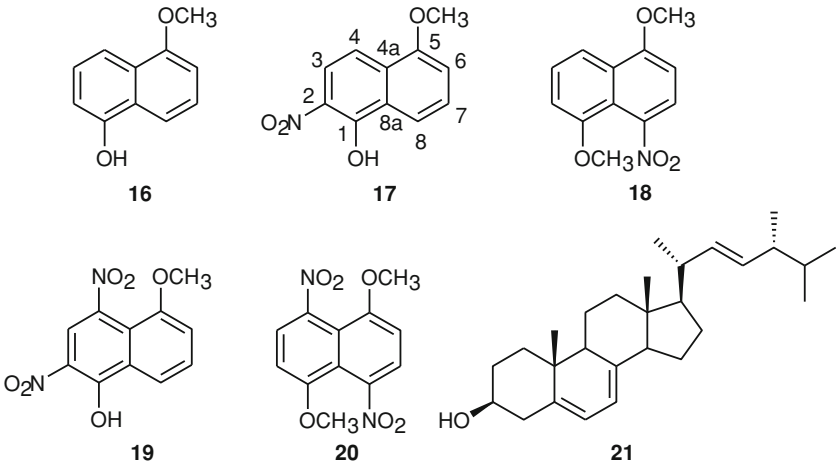


Fig. 8.2 Structures of naphthol (**16**), nitronaphthalenes **17**–**20**, and ergosterol (**21**)

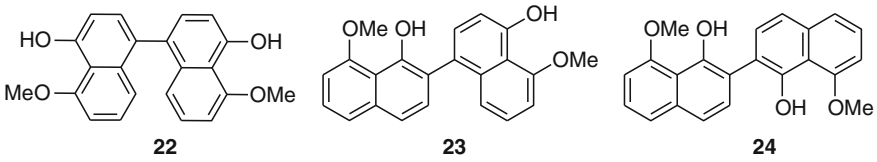


Fig. 8.3 Dimeric naphthols **22**–**24**

The very simple 1,8-dihydroxynaphthalene **28** is biosynthetically converted into a dimer, named palmarumycin CP_1 (**29**) after the producing strain, *Coniothyrium palmarum* (Krohn et al. 1994a) for biosynthetic studies see (Bode et al. 2000; Bode and Zeeck 2000). A related cyclization could be performed chemically with silver oxide as the oxidant (Krohn et al. 1997a). This initial metabolite **29** is further modified by different fungi into an enormous variety of more than a hundred different biologically highly active metabolites. The occurrence, isolation, structure elucidation, and biology are covered in two recent reviews (Krohn 2003; Cai et al. 2010). Figure 8.4 only shows a very small selection of the metabolites, i.e., the bridged palmarumycin CP_1 (**30**) (Krohn et al. 1994a) and related spirodioxynaphthalenes, also named palmarumycins (Krohn et al. 1994b, 1997b; Dai et al. 2007), the monoepoxide Sch 49211 (**31**) and other derivatives isolated by the Schering group (Chu et al. 1994, 1995, 1996), the diepoxides such as **32** (Schlingmann et al. 1993), the chlorinated palmarumycin CP_4 (**33**) (Krohn et al. 1994a) or the hydroxylated derivatives cladosporin H (**34**) (Baer and Hanna 1980) and decaspirone (**35**) (Jiao et al. 2006) (Fig. 8.5).

Many of the derivatives have also been synthesized employing the biomimetic oxidation (Wipf and Jung 1998; Inoue et al. 2003; Wipf and Lynch 2003; Rodríguez and Wipf 2004) or the Diels–Alder approach (Ragot et al. 1999; Coutts et al. 2000; Barrett et al. 2002; Krohn et al. 2010; Aslan et al. 2011).

Related, biologically active spirobisnaphthalenes, the preussomerins A–F, coupled by three oxygen bridges, were initially discovered by Gloer et al. (Weber et al. 1990; Weber and Gloer 1991). Later, we also isolated the antifungal metabolites preussomerins J, K, and L from an endophytic fungus, a Mycelia sterilia, from the root of *Atropa belladonna* (Krohn et al. 2001a) (Fig. 8.6). The

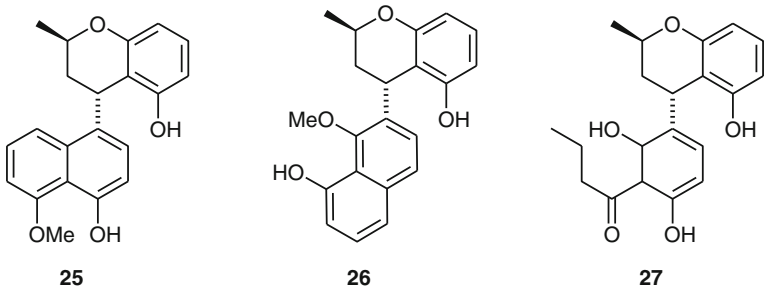


Fig. 8.4 Dimers isolated from a *Nodulisporium* sp. (Dai et al. 2009)

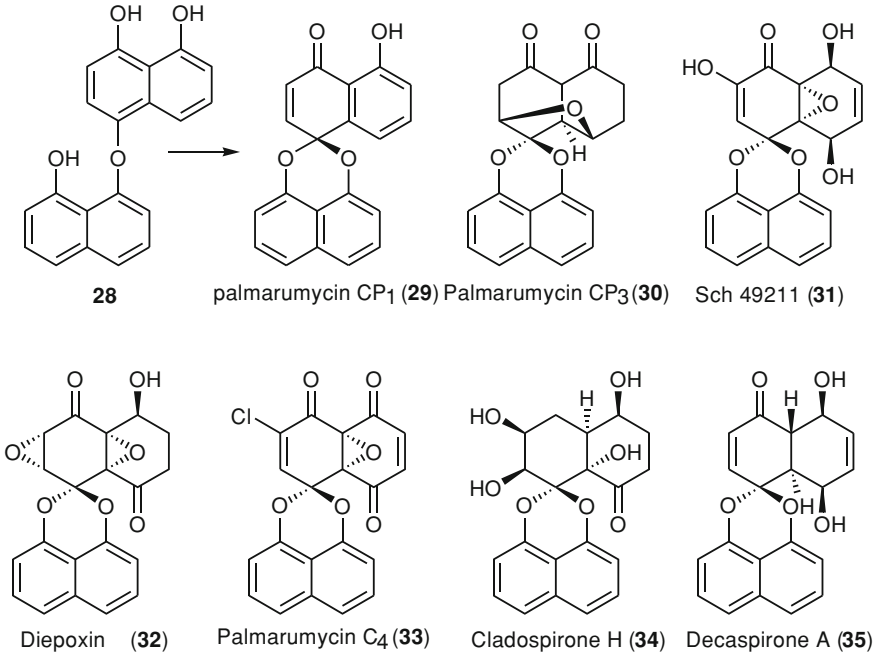


Fig. 8.5 Selection of spirobisnaphthalenes

culture extract of the fungus was found to have antibacterial and antifungal activity toward early successional coprophilous fungi. However, the activity against the fungi *M. violaceum* and *Eurotium repens* is relatively moderate compared to that of related preussomerins A–F against coprophilous fungi as determined by Weber and Gloer (1991) and Weber et al. (1990).

In 1995, we discovered highly antifungal biaryl ethers isolated from cultures of *Phomopsis* sp., the phomosines A–C (**39**, **40,** and **41**) (Krohn et al. 1995) (Fig. 8.7). Later, we isolated further derivatives, the phomosines D–G (Dai et al. 2005) and H–J (Krohn et al. 2011) and further analogs from another *Phomopsis* sp. with variation at the aldehyde function and on the benzoic acid group (Ahmed et al. 2011).

All isolated substances showed moderate fungicidal and antibacterial activity against four fungal and two bacterial test organisms. Almost 40 derivatives were prepared semisynthetically, changing mostly the aldehyde and the phenolic groups (Krohn et al. 2012). However, the very good antifungal properties of the natural product **39** have not yet been able to be improved.

Replacing one carbon by one oxygen atom in the naphthalene skeleton leads to the isochromene and isochromanone (isocoumarin) group of antifungal natural products. These mostly ketide-derived (Hill 1986; Pontius et al. 2008) natural products are very often found in fungi and have been known for many years. We first isolated mellein and derivatives from an endophytic *Pezicula* sp. (Schulz et al. 1995).

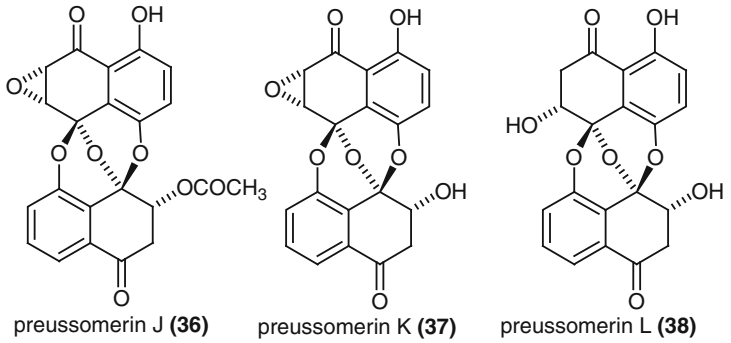


Fig. 8.6 Structures of preussomerins J–L

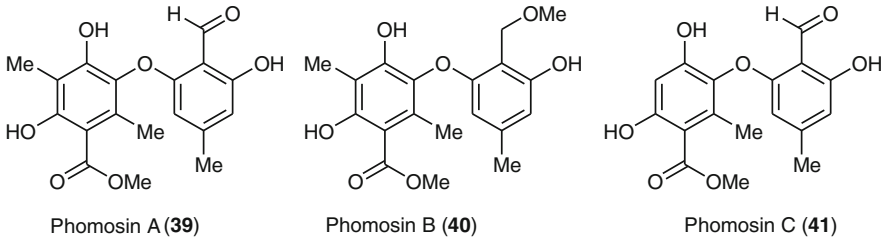


Fig. 8.7 Antifungal biaryl ethers from *Phomopsis* sp

Later, in addition to known mellein derivatives, we found many such compounds, for instance **42**–**44** from *Plectophomella* sp. (Krohn et al. 1997c) (Fig. 8.8). Others were isolated from the culture extract of an endophytic Mycelia sterilia from the Canadian thistle, *Cirsium arvense* (Krohn et al. 2001b), or from an endophytic *Scytalidium* sp., which had been isolated from a leaf of a *Salix* species growing in the Harz Mountains in Lower Saxony, Germany (Krohn et al. 2004). An unidentified endophyte from *Erica arborea* from Gomera also produced isocoumarins (Hussain et al. 2007). Crude ethyl acetate culture extracts, both from fungi grown on malt-soya and biomalt semi-solid agar culture media, were active in the agar diffusion assays against *E. coli*, *M. violaceum*, *E. repens*, *Mycotypha microspora,* and inhibited the growth of the alga, *C. fusca,* and the plant, *Lemna* (Krohn et al. 2004). More recently, we also

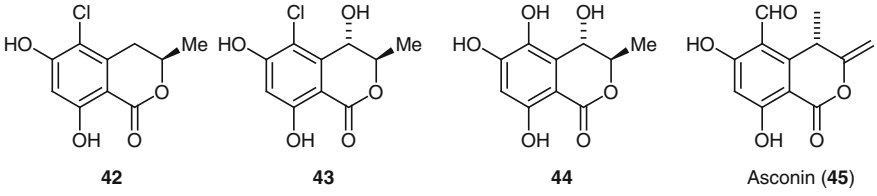


Fig. 8.8 A selection of metabolites from the antifungal class of isocoumarin natural products

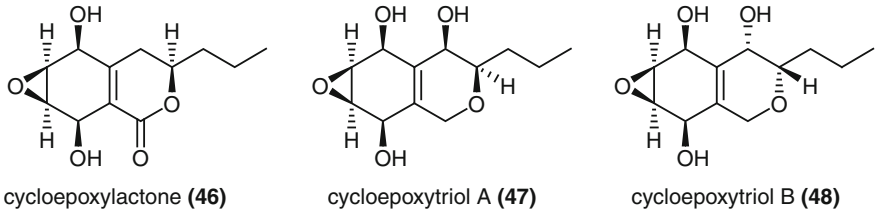


Fig. 8.9 Cycloepoxylactone (**46**), cycloepoxytriol A and B (**47** and **48**)

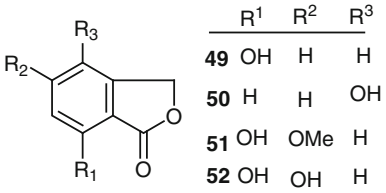
isolated isocoumarin derivatives from a *Phomopsis* sp. along with biaryl ethers (Dai et al. 2005; Ahmed et al. 2011) from a *Seimatosporium* sp. (Hussain et al. 2011a).

The highly active unsaturated asconin (**45**) was isolated from the endophytic fungus *Ascochyta* sp. from *Melilotus dentatu*s (Krohn et al. 2007a). The absolute configuration was assigned by a combination of X-ray analysis, solid-state CD, and TDDFT calculations (review: Pescitelli et al. 2009).

Hydrogenation of the aromatic ring in chromanones leads to the creation of new functionalities. For instance, a remaining double bond can be epoxygenated to yield highly chemically and biologically active 2,3-epoxycyclohexanes. The endophytic fungus, *Phomopsis* sp., from *Laurus azorica* produced such new, bioactive 2,3-epoxycyclohexanes, cycloepoxylactone (**46**), cycloepoxytriol A and B (**47** and **48**) (Fig. 8.9), together with known isocoumarins (Hussain et al. 2009a) Cycloepoxylactone (**46**) showed good antibacterial, antifungal, and algicidal activities against *B. megaterium*, *M. violaceum*, and *C. fusca*, respectively, whereas cycloepoxytriol B (**48**) had good algicidal activity against *C. fusca*. Cycloepoxytriol A (**47**) was inactive in these tests (Hussain et al. 2009a).

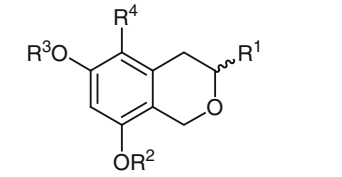
Replacing the six-membered ring in the isocoumarin skeleton by a five-membered ring leads to benzofuranes. These compounds **49–52** (Fig. 8.10) retained antifungal activity and were isolated from the endophytic fungus *Melilotus dentatus.* Compounds **49–51** were tested for herbicidal, antibacterial, and antifungal activities. Compounds **50** and **51** showed good algicidal activity against the alga *C. fusca* and also antifungal activity against *M. violaceum* (Hussain et al. 2009b).

Absence of the lactone carbonyl group in the isocoumarins of type **42** leads to benzopyrans (isochromanes). Pseudoanguillosporin A (**53**) and B (**56**), two new isochromanes, were isolated from the endophytic fungus *Pseudoanguillospora* sp. (Kock et al. 2009) (Fig. 8.11)

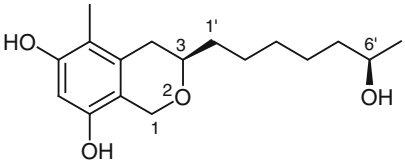


	R^1	R^2	R^3
49	OH	H	H
50	H	H	OH
51	OH	OMe	H
52	OH	OH	H

Fig. 8.10 Benzofuranes isolated from *Meliotus dentatus*



	R^1	R^2	R^3	R^4	C-3
53	*n*-heptyl	H	H	CH_3	*R*
54	CH_3	CH_3	CH_3	H	*S*
55	CH_3	H	H	CH_3	*R*



(3*R*,6'*R*)-**56**

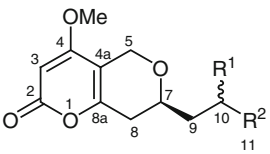
Fig. 8.11 Pseudoanguillosporin **53–56** from the endophytic fungus *Pseudoanguillospora* sp. (Kock et al. 2009)

The antibacterial, fungicidal, and algicidal properties of **53** and **56** were tested in comparison to four standard antibiotics both in an agar diffusion assay and in a microtiter assay in liquid culture. Although all of the compounds were biologically active, the strongest antifungal inhibitions were caused by **53**. Substances **53** and **56** were antibacterial against the Gram-positive bacterium *B. megaterium*. The two compounds were both active in assays against the fungal-like Oomycete, *P. infestans*, **53** causing the greatest inhibition (Kock et al. 2009).

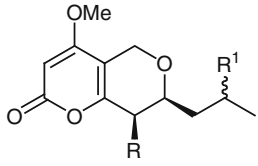
The presence of two oxygen atoms in the bicyclic skeleton leads to another group of strongly antifungal metabolites, the 7,8-dihydropyrano[4,3-*b*]pyran-2(5H)-ones, generally named pyranocines. Two representatives, pyranocines **57** and **58,** were isolated from an endophytic *Phomopsis* sp. (Hussain et al. 2012).

The isolated compounds **57** and **58** (Fig. 8.12) were tested in an agar diffusion assay for their antifungal, antibacterial, and algicidal properties toward *Botrytis cinerea*, *S. tritici, P. infestans*, *M. violaceum, E. coli, B. megaterium*, and *C. fusca*. Both compounds showed not only good antifungal toward *S. tritici* and *M. violaceum*, but also antibacterial and algicidal properties against *E. coli, B. megaterium*, and *C. fusca* (Hussain et al. 2012).

Four new pyrenocines, phomopsinones **59–62** (Fig. 8.12), were isolated from an endophytic strain of *Phomopsis* sp., which had colonized the halotolerant plant, *Santolina chamaecyparissus,* from Sardinia (Hussain et al. 2011a).



57 R = H, R^1= R^2 = H
58 R = H, R^1 = "=O" (keto), R^2 = CH_3



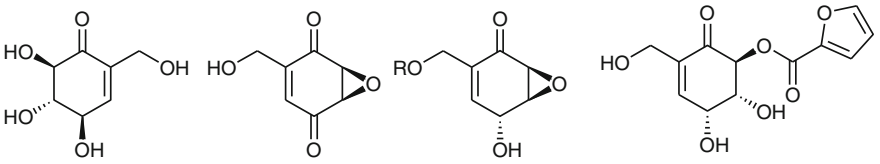
59: R = R^1 = H
60: R = H, R^1 = β-OH
61: R = H, R^1 = α-OH
62: R = OH, R^1 = H

Fig. 8.12 New pyranocines **57–62**. (61,62)

Compounds **59–62** were tested in an agar diffusion assay for their antifungal activity toward *B. cinerea*, *P. oryzae*, and *S. tritici*, whereby compound **59** showed very strong antifungal activity against *B. cinerea*, *P. oryzae,* and *S. tritici*, and **62** showed good activity against *B. cinerea* and *S. tritici,* comparable to that of the control substances. Compound **60** had moderate antifungal, antibacterial, and algicidal properties toward *P. oryzae*, *S. tritici, M. violaceum, E. coli, B. megaterium*, and *C. fusca.* Similarly, **61** showed moderate antifungal, antibacterial, and algicidal properties toward *M. violaceum, E. coli, B. megaterium*, and *C. fusca* and good antifungal activity against *B. cinerea* and *P. oryzae* (Hussain et al. 2011a).

When investigating the fungus *Phomopsis* sp., isolated from the plant *Notobasis syriaca*, the culture extract showed excellent fungicidal activity, particularly against *P. infestans*, as well as moderate algicidal and bacterial activities (Hussain et al. 2011b). From this fungus, we isolated new and known derivatives of the monocyclic, but highly active, epoxidone derivatives 2-hydroxymethyl-4β,5α,6β-trihydroxycyclohex-2-en (**63**), (-)-phyllostine (**64**), (+)-epiepoxydon (**65**), and (+)-epoxydon monoacetate (**66**) (Hussain et al. 2011b, c).

The isolated compounds **63–66** were tested in an agar diffusion assay for their antifungal, antibacterial, and algicidal properties toward *M. violaceum, E. coli, B. megaterium*, and *C. fusca*. Metabolite **63** was also tested for antibacterial activity in a MIC (minimum inhibitory concentration)-assay in liquid medium against *Legionella pneumophila* Corby, *E. coli* K12, and *B. megaterium.* All of the tested metabolites were antibacterial against at least one of the test organisms, metabolite **63** even at a concentration of 25 μg/ml against *L. pneumophila.* The metabolites were also antifungal (Hussain et al. 2011b, c).



A number of other epoxidone derivatives were found in the culture extract of an endophytic *Phoma* sp., isolated from the plant *Salsola oppositifolia*. One new derivative was named epoxydine B (**67**) (Qin et al. 2009a, b). Compound **67** was also antifungal against *M. violaceum* (Qin et al. 2009a, b).

The interesting groups of massarilactones and massarigenins were initially isolated by Gloer et al. from the freshwater aquatic fungus, *Massarina tunicate* (Oh et al. 2001, 2003) and also synthesized by Snider and coworker (Gao and Snider 2004) and Gao et al. (2003). In our group, we isolated in addition to the known massarilactone A (**68**), the related derivatives massarilactones C (**69**) and D (**70**) from the endophytic fungus *Coniothyrium* sp. from *Carpobrotus edulis* (Kock

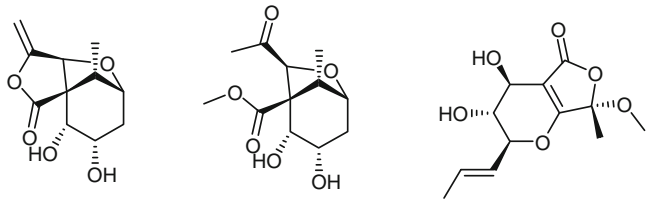


Fig. 8.13 Structures of selected massarilactones

et al. 2007) and others (massarilactones E–G) from *Coniothyrium* sp. that was associated with the plant *Artemisia maritime* (Krohn et al. 2007b). The absolute configuration of massarigenin A from *Microsphaeropsis* sp. was elucidated by the "solid-state circular CD/TDDFT" method (Hussain et al. 2011d) (Fig. 8.13). The massarilactones proved to be of only moderate antifungal activity.

In contrast, the bicyclic exomethylene furofuranones **71–75**, derivatives of discosiolide (**71**) that were isolated from *Sporothrix* sp., *Discosia* sp,. and *Pezzicula livida* all showed good activity against *M. violaceum* and *E. repens* (Krohn et al. 1994c) (Fig. 8.14).

Tricyclic oxygen heterocycles, the pestalotheols A–D were first isolated from the endophytic fungus *Pestalotiopsis theae* by Li et al. (2008). Later, in our group, we isolated new derivatives, the pestalotheols E–G (**76–78**) from an unidentified Ascomycete, isolated from the tree, *Arbutus unedo* (Qin et al. 2011) (Fig. 8.15). Compounds **76–78** were tested in an agar diffusion assay for their antifungal, antibacterial, and algicidal properties toward *M. violaceum, E. coli, B. megaterium*, and *C. fusca,* respectively; all the metabolites inhibited all four test organisms (Qin et al. 2011).

Other tricyclic dioxygen heterocycles **80–83,** but incorporating the isocoumarin skeleton, were isolated from *Microdochium bolleyi*, an endophytic fungus from *Fagonia cretica*, a herbaceous plant of the semi-arid coastal regions of Gomera, together with the known monocerin (**79**) (Zhang et al. 2008a, b) (Fig. 8.16).

Compounds **79, 81,** and **82** showed good antifungal, antibacterial, and antialgal activities against *M. violaceum, E. coli, B. megaterium,* and *C. fusca*. Compound **80** was only moderately antifungal and antialgal (Zhang et al. 2008a, b).

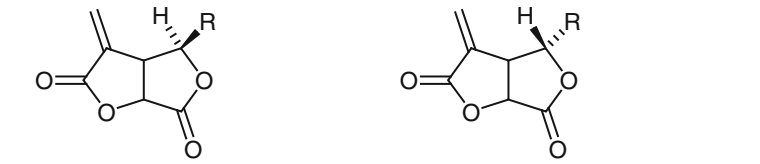


Fig. 8.14 Structures of bicyclic exomethylene furofuranones **71–75**

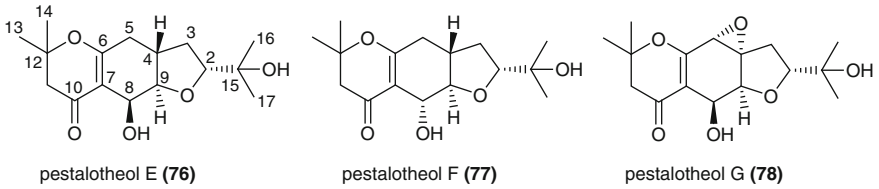


Fig. 8.15 Structures of pestalotheols E–G (**76**–**78**)

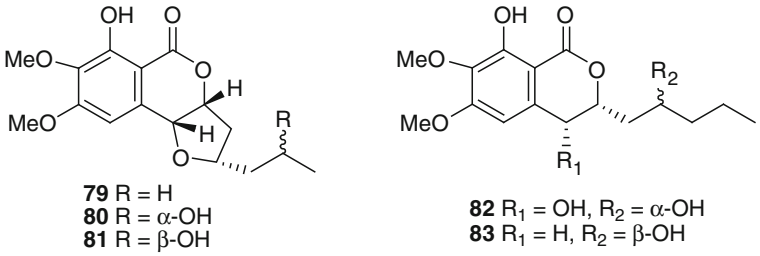


Fig. 8.16 Tricyclic and substituted isocoumarins **79**–**83**

Very often, relatively simple lactones show good antifungal activity. For instance, the pyrenocines (citreopyrones) **84** and **85**, isolated from an unidentified endophytic fungus, which had been isolated from the plant, *Trifolium dubium*, exhibited good antifungal and algicidal activities (Krohn et al. 2008b) (Fig. 8.17).

Bicyclic lactones, named fusidilactones (**86**), were isolated from the endophytic *Fusidium* sp., an endophyte in the leaves of *Mentha arvensis*, growing in a meadow near Hahausen, Lower Saxony, Germany. The compound showed good activity against the fungus *M. violaceum* (Krohn et al. 2002; Qin et al. 2009a, b).

A number of antifungal polycyclic lactones are oxidation products of terpenoids rather than of polyketide origin. For example, the oidiolactones are metabolites of *Oidiodendron truncata*. The fungus was isolated from an extreme location: the top of Enlang mountain (4,000 m) in China. Oidiolactone **87** was strongly antifungal against *M. violaceum*.

Similarly, a number of novel botryane (**88**) metabolites (Fig. 8.18) were isolated from the fungus *Geniculosporium* sp., which had been associated with the red alga, *Polysiphonia* sp. The botryanes showed inhibitory activity against the test organisms used in these studies: *C. fusca*, *B. megaterium,* and *M. violaceum.* The fungicidal and antibacterial activities could play a role in the association by defending the host from microbial pathogens (Krohn et al. 2005).

Thirteen new metabolites, named oblongolides were found in the culture media of an endophytic strain of *Phomopsis* sp., which had colonized the halotolerant plant, *Melilotus dentatus,* from the shores of the Baltic Sea, near Ahrenshoop, Germany. These new botryane metabolites (for instance **89**–**90**) were all biologically active against some or all of the test organisms: the gram-positive bacterium, *B. megaterium,* and the fungi, *M. violaceum* and *S. tritici.*

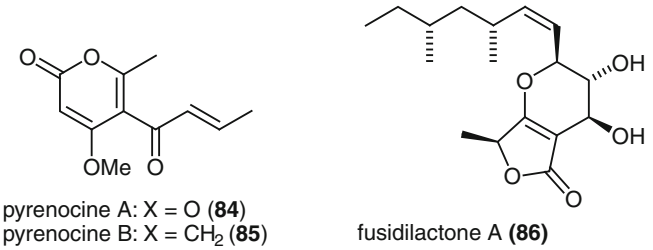


Fig. 8.17 Structures of pyrenocine A (**84**) and B (**85**) and fusidilactone A (**86**)

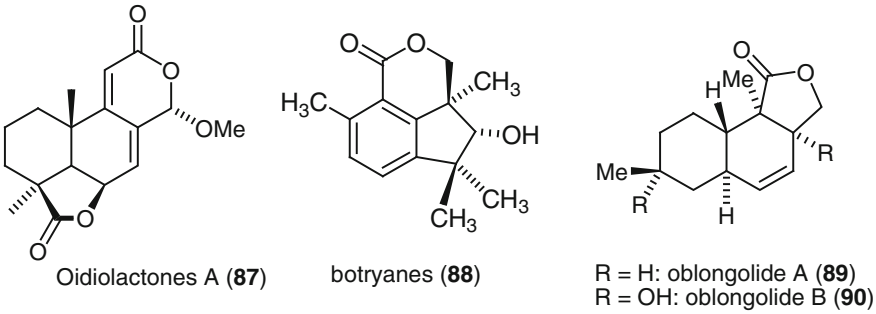


Fig. 8.18 The oidiolactones, botryanes and oblongolides

Interesting structures are displayed in the spirodioxo compound dinemasones A (**91**) and the condensed bicyclic bispyrane dinemasones B (**92**) (Fig. 8.19). The compounds were in the culture extracts of the endophytic fungus, *Dinemasporium strigosum,* which had grown in the roots of *Calystegia sepium*. Both compounds were very active against the fungus *M. violaceum* (Krohn et al. 2008c).

Other tricyclic oxygen heterocycles are represented by xanthones, a large and biologically highly active class of compounds. Only a few representatives from

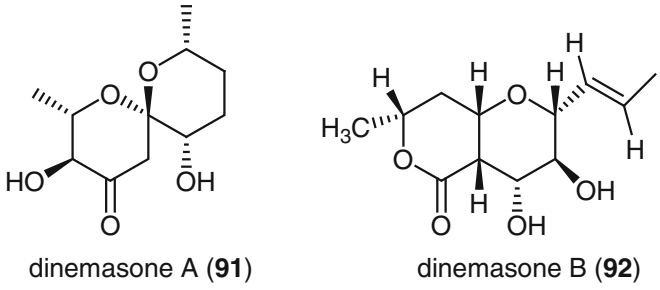
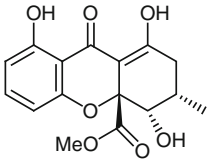
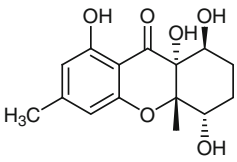


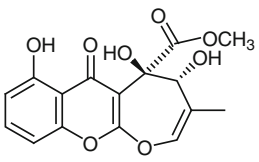
Fig. 8.19 Dinemasones A (**91**) and B (**92**)



Blennolide A (**93**)



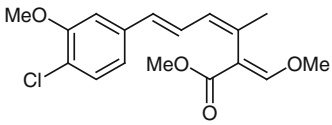
diversonol (**94**)



microsphaeropsone A (**95**)

Fig. 8.20 Some representative xanthones: blennolide A (**93**), diversonol (**94**), and microsphaeropsones A (**95**)

Fig. 8.21 Structure of strobilurin G (**96**)



Strobilurin G (**96**)

our research can be shown here; the topic has recently been covered in a review by Masters and Bräse (2012). Blennolide A (**93**) is a monomer of the known dimeric secalonic acid and was isolated from *Blennoria* sp. (Zhang et al. 2008a, b). A widely distributed xanthone is diversonol (**94**), the key starting material for a plethora of derivatives, isolated in our group from a *Microdiplodia* sp.(Siddiqui et al. 2011) Structurally very interesting is the oxepino[2,3-*b*]chromen-6-one derivative microsphaeropsone A (**95**) (Fig. 8.20), isolated from a *Microsphaeropsis* sp. (Krohn et al. 2009). All of these xanthones are antifungal.

Last but not least, we shall mention the strobilurins, which are the lead compounds for perhaps the most important antifungal drugs today (Sauter et al. 1999). The strobilurins were originally isolated by Anke et al. (1977) from the Basidiomycete, *Strobilurus tenacellus*. The active principle is the (*E*)-*β*-methoxyacrylate unit. We isolated strobilurin G (**96**) from the culture medium of a fungus found in the soil found near Richards Bay in South Africa (Krohn et al. 2001c) (Fig. 8.21).

8.2 Conclusion

Almost all plants that have been studied to date are colonized by endophytic fungi, and approximately half of these fungal endophytes produced antifungal metabolites (Schulz et al. 1999). These metabolites belong to diverse structural groups, and an unusually high proportion of the structures is novel. However, there have only been very few studies that have investigated whether the antifungal compounds isolated from plants were in effect those synthesized by their fungal inhabitants. In the future, when searching for antifungal plant metabolites, chemists and biologists should also consider whether these are in effect fungal metabolites.

References

Ahmed I, Hussain H, Schulz B, Draeger S, Padula D, Pescitelli G, van Ree T, Krohn K (2011) Three new antimicrobial metabolites from the endophytic fungus, *Phomopsis* sp. Eur J Org Chem, pp 2867–2873

Anke T, Oberwinkler F, Steglich W, Schramm G (1977) J Antibiot 30:806–810

Aslan A, Altun S, Ahmad I, Flörke U, Schulz B, Krohn K (2011) Synthesis of biologically active nonnatural palmarumycin derivatives. Eur J Org Chem 6:1176–1188

Baer H, Hanna ZS (1980) Palladium-catalyzed, allylic aminations and alkylations of an unusual sugar acetate. Carbohydr Res 78:C11–C14

Baker DD, Chu M, Oza U, Rajgarhia V (2007) The value of natural products to future pharmaceutical discovery. Nat Prod Rep 24:1225–1244

Barrett AGM, Blaney F, Campbell AD, Hamprecht D, Meyer T, White AJP, Witty D, Williams DJ (2002) A unified route to the palmarumycin and preussomerin natural products: an enantioselective synthesis of (-)-preussomerin G. J Org Chem 67:2735–2750

Bode HB, Zeeck A (2000) UV mutagenesis and enzyme inhibitors as tools to elucidate the late biosynthesis of the spirobisnaphthalenes. Phytochemistry 55:311–316

Bode HB, Wegner B, Zeeck A (2000) Biosynthesis of cadospirone bisepoxide, a member of the spirobisnaphthalene family. J Antibiot 53:153–157

Butler MS (2004) The role of natural product chemistry in drug discovery. J Nat Prod 67:2141–2153

Cai Y-s, Guo Y-W, Krohn K (2010) Structure, bioactivities, biosynthetic relationships and chemical synthesis of the spirodioxynaphthalenes. Nat Prod Rev 27:1840–1870

Chu M, Truumees I, Patel MG, Gullo VP, Puar MS, McPhail AT (1994) Structure of Sch 49209: a novel antitumor agent from the fungus *Nattrassia mangiferae*. J Org Chem 59:1222–1223

Chu M, Truumees I, Patel MG, Blood C, Das PR, Puar MS (1995) Sch 50673 and Sch 50676, two novel antitumor fungal metabolites. J Antibiot 48:329–331

Chu M, Patel MG, Pai J-K, Das J-K, Puar MS (1996) Sch 53823 and Sch 53825, Novel fungal metabolites with phospholipase D inhibitory activity. Bioorg Med Chem Lett 6:579–584

Coutts IGC, Allcock RW, Scheeren HW (2000) Novel synthetic approaches to the palmarumycin skeleton. Tetrahedron Lett 41:9105–9107

Dai J, Krohn K, Flörke U, Gehle D, Aust H-J, Draeger S, Schulz B, Rheinheimer K (2005) Novel highly substituted biaryl ethers, phomosines D-G, isolated from the endophytic fungus *Phomopsis* sp. from *Adenocarpus foliolosus*. Eur J Org Chem, pp 5100–5105

Dai J, Krohn K, Elsässer B, Flörke U, Draeger S, Schulz B, Pescitelli G, Salvadori P, Antus S, Kurtán T (2007) Metabolic products of the endophytic fungus *Microsphaeropsis* sp. from *Larix decidua*. Eur J Org Chem, pp 4845–4854

Dai J, Krohn K, Draeger S, Schulz B (2009) New naphthalen-chroman coupling products from the endophytic fungus *Nodulisporium* sp. from *Erica arborea*. Eur J Org Chem, pp 1564–1569

Dreyfuss MM, Chapela IH (1994) Potential of fungi in the discovery of novel low-molecular weight pharmaceuticals. In: Gullo VP (ed) The discovery of natural products with therapeutic potential, vol 3. Butterworth-Heinemann Butterworth-Heinemann, Boston, pp 49–80

Gao X, Snider BB (2004) Syntheses of (-)-TAN-2483A, (-)-Massarilactone B, and the fusidilactone B ring system. Revision of the structures of and syntheses of (±)-Waol A (FD-211) and (±)-Waol B (FD-212). J Org Chem 69:5517–5527

Gao X, Nakadai M, Snider BB (2003) Synthesis of (-)-TAN-2483A. Revision of the structures and syntheses of (±)-FD-211 (Waol A) and (±)-FD-212 (Waol B). Org Lett 5:451–454

Hill RA (1986) Naturally occurring isocoumarins. Prog Chem Nat Prod 49:1–78

Hussain H, Krohn K, Ullah Zia, Draeger S, Schulz B (2007) Bioactive chemical constituents of two endophytic fungi. Biochem Syst Ecol 35:898–900

Hussain H, Akhtar N, Draeger S, Schulz B, Pescitelli G, Salvadori P, Antus S, Kurtán T, Krohn K (2009a) New bioactive 2,3-epoxycyclohexenes and isocoumarins from the endophytic fungus *Phomopsis* sp. from *Laurus azorica*. Eur J Org Chem, pp 749–756

Hussain H, Krohn K, Draeger S, Meier K, Schulz B (2009b) Bioactive chemical constituents of a sterile endophytic fungus from *Meliotus dentatus*. Rec Nat Prod 3:114–117

Hussain H, Ahmed I, Schulz B, Draeger S, Flörke U, Pescitelli G, Krohn K (2011a) Solid-state circular dichroism and hydrogen bonding: absolute configuration of Massarigenin A from *Microsphaeropsis* sp. Chirality 23:617–623

Hussain H, Krohn K, Ahmed I, Draeger S, Schulz B, Di Pietro S, Pescitelli G (2011b) Phomopsinones A-D: four new pyranocines from endophytic fungus *Phomopsis* sp. J Nat Prod 74:365–373

Hussain H, Krohn K, Tichimene MK, Ahmed I, Meier K, Steinert M, Draeger S, Schulz B (2011c) Antimicrobial chemical constituents from the endophytic fungus *Phomopsis* sp. from *Notobasis syriaca*. Nat Prod Commun 6:1905–1909

Hussain H, Schulz B, Draeger S, Krohn K (2011d) Two new antimicrobial metabolites from the endophytic fungus, *Seimatosporium* sp. Nat Prod Commun 7:293–294

Hussain H, Ahmed I, Schulz B, Draeger S, Krohn K (2012) Four new antimicrobial pyranocines from the endophytic fungus, *Phomopsis* sp. Fitoterapia 83:523–526

Inoue M, Nabatame K, Hirama M (2003) A novel route to 1,8-dihydroxynaphthalene-derived natural products: synthesis of (+-)-CJ-12,372. Heterocycles 59:87–92

Jiao P, Swenson DC, Gloer JB, Campbell J, Shearer CA (2006) Decaspirones A-E, bioactive spirodioxynaphthalenes from the freshwater aquatic fungus *Decaisnella thyridioides*. J Nat Prod 69:1667–1771

Kock I, Draeger S, Schulz B, Elsässer B, Kurtán T, Kenéz Á, Antus S, Pescitelli G, Salvadori P, Speakman J-B, Rheinheimer J, Krohn K (2009) Pseudoanguillosporin A and B, two new isochromans isolated from the endophytic fungus *Pseudoanguillospora* sp. Eur J Org Chem, pp 1427–1434

Kock I, Krohn K, Egold H, Draeger S, Schulz B and Rheinheimer J (2007) New massarilactones, massarigenins and coniothyrenol, isolated from the endophytic fungus *Coniothyrium* sp. from *Carpobrotus edulis*. Eur J Org Chem 2186–2190

Krohn K (2003) Natural products derived from naphthalenoid precursors by oxidative dimerization. In: Herz W, Falk H, Kirby GW, Moore RE, Tamm Ch (eds) Progress in the chemistry of organic natural products, vol 85., SpringerWien, New York, pp 1–49

Krohn K, Aslan A (2009) Synthesis of daldinol and nodulisporin A by oxidative dimerisation of 8-methoxynaphthalen-1-ol. Nat Prod Commun 4:87–88

Krohn K, Schulz B (2011) Isolation of metabolites from the fungal culture broth: structural diversity of bioactive secondary metabolites from fungi. In: Pirttilä AM, Sorvari S (eds) Prospects and applications for plant-associated microbes. A laboratory manual, Part B: Fungi, pp 113–117, 237–254

Krohn K, Franke C, Jones PG, Aust H-J, Draeger S, Schulz B (1992) Wirkstoffe aus Pilzen, I. Isolierung, synthese und biologische Wirkung von Coniothyriomycin und analogen offenkettigen Imiden. Liebigs Ann Chem 1992:789–798

Krohn K, Michel A, Flörke U, Aust H-J, Draeger S, Schulz B (1994a) Biologically active metabolites from fungi, 4. Palmarumycins CP_1 to CP_4 from *Coniothyrium palmarum*: isolation, structure elucidation and biological activity. Liebigs Ann Chem 1994:1093–1097

Krohn K, Michel A, Flörke U, Aust H-J, Draeger S, Schulz B (1994b) Biologically active metabolites from fungi, 5. Palmarumycins C_1 to C_{16} from *Coniothyrium* sp.: isolation, structure elucidation, and biological activity. Liebigs Ann Chem 1994:1099–1108

Krohn K, Ludewig K, Aust H-J, Draeger S, Schulz B (1994c) Biologically active metabolites from fungi, 3. Sporothriolide, discosiolide, and 4-epi-ethiosolide: new furofuranones from *Sporothrix* sp., *Discosia* sp. and *Pezzicula livida*. J Antibiot 46:113–118

Krohn K, Michel A, Roemer E, Flörke U, Aust H-J, Draeger S, Schulz B, Wray V (1995) Biologically active metabolites from fungi, 6. Phomosines A-C: three new biaryl ethers from *Phomopsis* sp. Nat Prod Lett 9:309–314

Krohn K, Beckmann K, Aust H-J, Draeger S, Schulz B, Busemann S, Bringmann G (1997a) Biologically active metabolites from fungi, 10: generation of the palmarumycin spiroacetal

framework by oxidative cyclization of an open chain metabolite from *Coniothyrium palmarum*. Liebigs Ann Chem 1997:2531–2534

Krohn K, Beckmann K, Flörke U, Aust H-J, Draeger S, Schulz B, Busemann S, Bringmann G (1997b) Biologically active metabolites from fungi, 9. New palmarumycins CP_{4a} and CP_5 from *Coniothyrium palmarum*: structure elucidation, crystal structure analysis and determination of the absolute configuration by CD-calculations. Tetrahedron 53:3101–3110

Krohn K, Bahramsari R, Flörke U, Ludewig K, Kliche-Spory C, Michel A, Aust H-J, Draeger S, Schulz B, Antus S (1997c) Biologically active metabolites from fungi, 8. dihydroisocoumarins from fungi: isolation, structure elucidation, circular dichroism, enantioselective synthesis, and biological activity. Phytochemistry 45:313–320

Krohn K, Flörke U, John M, Root N, Steingröver K, Aust H-J, Draeger S, Schulz B, Antus S, Simonyi M, Zsila F (2001a) Biologically active metabolites from fungi, 16. New preussomerins J, K and L from an endophytic fungus: structure elucidation, crystal structure analysis and determination of absolute configuration by CD calculations. Tetrahedron 57:4343–4348

Krohn K, Flörke U, Rao MS, Steingröver K, Aust H-J, Draeger S, Schulz B (2001b) Metabolites from fungi 15. New isocoumarins from an endophytic fungus isolated from the Canadian thistle *Cirsium arvense*. Nat Prod Lett 15:353–361

Krohn K, John M, Aust H-J, Draeger S, Schulz B (2001c) Biologically active metabolites from fungi 14: new antibacterial agent from a soil fungus. Nat Prod Lett 15:9–12

Krohn K, Biele C, Drogies K-H, Steingröver K, Aust H-J, Draeger S, Schulz B (2002) Biologically active secondary metabolites from fungi, 18. Fusidilactones, a new group of polycyclic lactones from an endophyte, *Fusidium* sp. Eur J Org Chem, pp 2331–2336

Krohn K, Elsässer B, Antus S, Kónya K, Ammermann E (2003) Synthesis and structure-activity relationship of antifungal coniothyriomycin analogues. J Antibiot 56:296–305

Krohn K, Sohrab MH, Aust H-J, Draeger S, Schulz B (2004) Biologically active metabolites from fungi 19: new isocoumarins and highly substituted benzoic acids from the endophytic fungus *Scytalidium sp*. Nat Prod Res 18:277–285

Krohn K, Dai J, Flörke U, Aust H-J, Draeger S, Schulz B (2005) Botryane metabolites from the fungus *Geniculosporium* sp. isolated from the marine red alga *Polysiphonia*. J Nat Prod 68:400–405

Krohn K, Kock I, Elsässer B, Flörke U, Schulz B, Draeger S, Pescitelli G, Antus S, Kurtán T (2007a) New natural products from the endophytic fungus *Ascochyta* sp. from *Meliotus dentatus*: configurational assignment by solid-state CD and TDDFT calculations. Eur J Org Chem, pp 1123–1129

Krohn K, Zia-Ullah Hussain H, Floerke U, Schulz B, Draeger S, Pescitelli G, Salvadori P, Antus S, Kurtán T (2007b) Massarilactones E-G, new bioactive metabolites from the endophytic fungus *Coniothyrium* sp., associated with the plant *Artimisia maritima*. Chirality 19:464–470

Krohn K, Kouam SF, Cludius-Brandt S, Draeger S and Schulz B (2008a) Bioactive nitronapthalenes from an endophytic fungus, *Coniothyrium* sp. and their chemical synthesis. Eur J Org Chem 3615–3618

Krohn K, Sohrab MH, Draeger S, Schulz B (2008b) New pyrenocines from an endophytic fungus. Nat Prod Commun 3:1689–1692

Krohn K, Sohrab MH, van Ree T, Draeger S, Schulz B, Antus S, Kurtán T (2008c) Dinemasones A, B and C, new bioactive metabolites from the endophytic fungus, *Dinemasporium strigosum*. Eur J Org Chem, pp 5638–5646

Krohn K, Kouam SF, Kuigoua GM, Hussain H, Cludius-Brand S, Flörke U, Kután T, Pescitelli G, Di Bari L, Draeger S, Schulz B (2009) Xanthones and new oxepino[2,3-*b*]chromones from three endophytic fungi. Chem Eur J 15:12121–12132

Krohn K, Wang S, Ahmed I, Altun S, Aslan A, Flörke U, Kock I, Schlummer S (2010) A new flexible route to palmarumycins CP_1, CP_2, and CJ-12.371 methyl ether. Eur J Org Chem, pp 4476–4481

Krohn K, Farooq U, Hussain H, Rheinheimer J, Draeger S, Schulz B, van Ree T (2011) Phomosines H-J, novel highly substituted biraryl ethers, isolated from the endophytic fungus *Phomopsis* sp. from *Ligustrum vulgare*. Nat Prod Commun 6:1907–1912

Krohn K, Hussain H, Egold H, Schulz B, Green I (2012) Chemical derivisation of phomosine A, a highly antifungal metabolite of *Phomopsis* sp. Arkivoc 2012:71–89

Li E, Tian R, Liu S, Chen X, Guo L, Che Y (2008) Pestalotheols A-D, bioactive metabolites from the plant endophytic fungus *Pestalotiopsis theae*. J Nat Prod 71:664–668

Masters K-S, Bräse S (2012) Xanthones from fungi, lichens and bacteria: the natural products and their synthesis. Chem Rev 112:3717–3776

Newman DJ, Cragg GM (2007) Natural products as sources of new drugs over the last 25 years. Nat Prod Rep 70:461–477

Oh H, Swenson DC, Gloer JB, Shearer CA (2001) Massarilactones A and B: novel secondary metabolites from the freshwater aquatic fungus *Massarina tunicata*. Tetrahedron Lett 42:975–977

Oh H, Swenson DC, Gloer JB, Shearer CA (2003) New bioactive rossigenin analogues and aromatic polyketide metabolites from the freshwater aquatic fungus *Massarina tunica*. J Nat Prod 66:73–79

Pearce C, Eckard P, Gruen-Wollny I, Hanske FG (2010) Microorganisms: their role in the discovery and development of medicines. In: Buss AD, Butler MS (eds) Natural product chemistry for drug discovery. The Royal Society of Chemistry, Cambridge, pp 215–244

Pescitelli G, Kurtán T, Flörke U, Krohn K (2009) Absolute structural elucidation of natural products: a focus on quantum-mechanical calculations of solid-state CD spectra. Chirality 21:S181–S201

Pontius A, Mohamed I, Krick A, Kehraus S, König GM (2008) Aromatic polyketides from marine algicolous fungi. J Nat Prod 71:272–274

Qin S, Krohn K, Flörke U, Schulz B, Draeger S, Pescitelli G, Salvadori P, Antus S, Kurtán T (2009a) Two new fusidilactones from a fungal endophyte, *Fusidium* sp. Eur J Org Chem, pp 3279–3284

Qin S., Krohn K, Schulz B (2009b) Two new metabolites, epoxydine A and B, from *Phoma* sp. Helv Chim Acta, pp 169–174

Qin S, Hussain H, Schulz B, Draeger S, Krohn K (2011) Pestalotheols E-H: antimicrobial metabolites from an endophytic fungus, from the tree *Arbutus unedo*. Eur J Org Chem, pp 5163–5166

Ragot JP, Steeneck C, Alcaraz M-L, Taylor RJK (1999) The synthesis of 1,8-dihydroxynaphthalene-derived natural products: palmarumycin CP_1 and CP_2, CJ-12,371, deoxypreussomerin A and novel analogues. J Chem Soc Perkin Trans 1:1073–1082

Rodríguez S, Wipf P (2004) Oxidative spiroacetalizations and spirolactonizations of arenes. Syntheis, pp 2767–2783

Sauter H, Steglich W, Anke T (1999) Strobilurine: evolution einer neuen Wirkstoffklasse. Angew Chem 111:1416–1438

Schlingmann G, West RR, Milne L, Pearce CJ, Carter GT (1993) Diepoxins, novel fungal metabolites with antibiotic activity. Tetrahedron Lett 34:7225–7228

Schulz B, Sucker J, Aust H-J, Krohn K, Ludewig K, Jones PG, Doering D (1995) Biologically active secondary metabolites of endophytic *Pezicula* species. Mycol Res 99:1007–1015

Schulz B, Römmert A-K, Dammann U, Aust H-J, Strack D (1999) The endophyte-host interaction: a balanced antagonism? Mycol Res 103:1275–1283

Schulz B, Boyle C, Draeger S, Römmert A-K, Krohn K (2002) Endophytic fungi: a source of novel biologically active secondary metabolites. Mycol Res 106:996–1004

Schulz B, Draeger S, delaCruz T, Rheinheimer J, Siems K, Loesgen S, Bitzer J, Schloerke O, Zeeck A, Kock I, Hussain H, Dai J, Krohn K (2008) Screening strategies for obtaining novel, biologically active, fungal secondary metabolites from marine habitats. Botanica Marina 51:219–234

Siddiqui IN, Zahoor A, Hussain H, Ahmed I, Ahmad VU, Padula D, Draeger S, Schulz B, Meier K, Steinert M, Kurtan T, Flörke U, Pescitelli G, Krohn K (2011) Two new diversonol and

blennolide derivatives from the endophytic fungus *Microdiplodia* sp.: absolute configuration of diversonol. J Nat Prod 74:365–373

Weber HA, Gloer JB (1991) The preussomerins: novel antifungal metabolites from the coprophilous fungus *Preussia isomera* Cain. J Org Chem 56:4355–4360

Weber HA, Baenziger NC, Gloer JB (1990) Structure of preussomerin A: an unusual new antifungal metabolite from the coprophilous fungus *Preussia isomera*. J Am Chem Soc 112:6718–6719

Wipf P, Jung J-K (1998) Total synthesis of palmarumycin CP1 and (+-)-deoxypreussomerin A. J Org Chem 63:3530–3531

Wipf P, Lynch SM (2003) Synthesis of highly oxygenated dinaphthyl ethers via SNAr reactions promoted by Barton's base. Org Lett 5:1155–1158

Zhang HW, Song YC, Tan RX (2006) Biology and chemistry of endophytes. Nat Prod Rep 23:753–771

Zhang W, Krohn K, Draeger S, Schulz B (2008a) Bioactive isocoumarins isolated from the endophytic fungus *Microdochium bolleyi*. J Nat Prod 71:1078–1081

Zhang W, Krohn K, Zia-Ullah, Flörke U, Pescitelli G, Di Bari L, Antus S, Kurtán T, Rheinheimer J, Draeger S and Schulz B (2008) New mono- and dimerc members of the secalonic acid family, blennolides A-G, isolated from fungus Blennoria sp. Chem Eur J 14: 4913–4923

Chapter 9
Combining Plant Essential Oils and Antimycotics in Coping with Antimycotic-Resistant *Candida* Species

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Abstract Resistance of microorganisms to antimicrobial agents is becoming a growing concern worldwide. Plant essential oils have potential in treatment of fungal infections not only due to direct antifungal activity but also because of their complex healing properties. Combining essential oils with antimycotics opens perspectives in increasing activity of both agents and also in prolonging use of antimycotics which are currently becoming less effective due to rising resistance of fungi. The aim of this chapter is to summarize studies on anti-*Candida* effect of combinations between essential oils and antimycotics, and to formulate prospects for future researches in this area. Analysis of published studies has shown that the most studied are combinations of essential oils with either amphotericin B or fluconazole in vitro. There is a lack of in vivo studies, and furthermore, even results of in vitro studies it is difficult to compare because of absence of uniform standard techniques for evaluation of antimycotic-essential oil combinations. These gaps should be filled in future researches. Moreover, future studies should be directed on antifungal activity of combinations between essential oil components and antimycotics, and between essential oils and other classes of antimicrobial agents, such as antiseptics, metallic nanoparticles, and quorum-sensing inhibitors.

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9.1 Introduction

Resistance to antimicrobial agents is a constantly progressive process which involves all types of antimicrobials, including also antimycotics (Anderson 2005). Although resistance to antifungal agents does not seem to be as much of a problem as resistance to antibiotics (Sanglard and Odds 2002), the number of effective antifungal classes is small. This is explained by the close location of humans and their fungal pathogens clades in the tree of life (Baldauf et al. 2000), and, because of this, there are few targets in fungal cells which are significantly different from potential targets in human cells. Antifungal drugs inhibit sterol biosynthesis of the membrane components (azoles, allylamines and morpholines), directly interact with the fungal cell membrane (polyenes) or affect cell wall biosynthesis (echinocandins) (Casalinuovo et al. 2004).

Most antifungal drugs target ergosterol and its biosynthesis. Ergosterol is the functional fungal analog of cholesterol in animal cells; it is found in the fungal cell membrane and takes part in modulation of membrane fluidity and as a signal for cell division. Ergosterol has different chemical properties from cholesterol and because of this is exploited as a target for antifungals such as polyenes (Amphotericin B). Amphotericin B is bound to ergosterol and is thought to form membrane-spanning channels with hydrophilic interiors (Matsumori et al. 2002). This leads to the leakage of essential components from the fungal cell and ultimately results in fungal cell death. Apart from ergosterol itself, in its synthesis there are several unique enzymatic steps that are chosen as targets for several other antifungal drugs, including terbinafine and the azoles (White et al. 1998). However, there are only few other targets in antifungal therapy other than ergosterol and its biosynthesis (Anderson 2005).

Resistance to antifungal drugs is commonly attributed to one of the following mechanisms: increased efflux of the drug from fungal cell by efflux pumps, alteration of structure or concentration of a target enzyme, or alteration of metabolism (Anderson 2005). Among mechanisms of *Candida* resistance to azoles the two most common ways are induction of the efflux pumps encoded by the MDR or CDR genes and acquisition of point mutations in the gene encoding for the target enzyme (ERG11). Resistance to echinocandins is caused by acquisition of point mutations in the FKS genes encoding the major subunit of target enzyme of the drug (Pfaller et al. 2012).

Despite more than 30 years of clinical use of amphotericin B, only minimal resistance has developed to this drug and it continues to be important in the treatment of a variety of fungal pathogens. However, some species of *Candida* including *C. lusitaniae, C. glabrata*, and *C. guilliermondii* are capable of expressing resistance to amphotericin B (Martins and Rex 1996). Because ergosterol is the target of polyene action, a change in its content, replacement with a different sterol, or reorientation of ergosterol in the membrane resulting in steric interference of the ergosterol-amphotericin B interaction are responsible for the decreased activity of this antifungal.

Azoles (fluconazole, itraconazole, ketoconazole, etc.) also target ergosterol biosynthesis; their target of action is a lanosterol demethylase enzyme, 14-α-demethylase, which is involved in the conversion of lanosterol to ergosterol. Another effect of these drugs is azole-induced accumulation of toxic 14-methyl sterols (Kelly et al. 1995). The three most commonly proposed mechanisms of azole resistance among *Candida* isolates are alteration of 14-α-demethylase, decreased accumulation of drugs inside the cell, and loss of function of the enzyme 5,6-desaturase. Resistance to azoles was reported in the late 1980s when resistant *C. albicans* was isolated after prolonged therapy with miconazole and ketoconazole (Casalinuovo et al. 2004). In recent time, fluconazole-resistant *C. albicans* strains and intrinsically resistant *Candida* species such as *C. glabrata* and *C. krusei*, have emerged in immunocompromised patients treated both for the therapy or prophylactic purposes (Martins et al. 1997; Krcmery and Barnes 2002).

Various studies have indicated increasing resistance to antifungal agents as an alarming sign of *Candida* nosocomial infections (Badiee and Alborzi 2011; Huang and Kao 2012; Pfaller 2012). Badiee and Alborzi (2011) evaluated minimal inhibitory concentrations (MICs) of different antimycotics against *Candida* species. The lower MIC90 was demonstrated by caspofungin (0.5 μg/ml) and amphotericin B (0.75 μg/ml), while for fluconazole MIC achieved 64 μg/ml. Multicenter prospective survey conducted in Spain reported the overall rather high susceptibility level of *Candida* spp., such as 77.6 % susceptibility rate for itraconazole and 91.9 % for fluconazole, but at the same time the study also demonstrated 24.1 % resistance rate to itraconazole and 14.5 % resistance to posaconazole in *C. glabrata*; furthermore, among *C. krusei* 81.5 % of isolates were resistant to itraconazole (Pemán et al. 2012).

The echinocandins are considered to be the first-line treatment of bloodstream infections caused by *C. glabrata*. However, in recent time resistance to these agents has also been emerged. Results of the two large surveillance programs, the SENTRY Antimicrobial Surveillance Program conducted in 2006–2010 and the Centers for Disease Control and Prevention population-based surveillance conducted in 2008–2010, demonstrated that a total of 162 isolates (9.7 %) among 1669 studied were resistant to fluconazole (MIC $\geq$ 64 μg/ml). Among fluconazole-resistant strains 98.8 % were nonsusceptible to voriconazole (MIC $>$ 0.5 μg/ml) and 9.3 % were resistant to caspofungin (Phaller et al. 2012). Strains of *C. albicans, C. glabrata,* and *C. krusei* with acquired resistance to echinocandins (caspofungin MIC $>$ 0.5 μg/ml) were also reported by the recent French study conducted in 2004–2010 (Dannaoui et al. 2012).

Increase in the rates of antifungal resistance explains increasing interest of scientists to alternative methods of antifungal treatment (Anderson 2005). Plant essential oils (EOs) have demonstrated high antifungal activity in many studies (Rai and Mares 2003; Pauli 2006; Palmeira-de-Oliveira et al. 2009). Their advantage compared with synthetic antimycotics is a broad spectrum of action (Reichling et al. 2009; Bassolé and Juliani 2012) including simultaneous activity not only against fungi but also against bacteria (Hyldgaard et al. 2012), viruses (Garozzo et al. 2011), and protozoans (Monzote et al. 2007), which is especially

important in mixed infections. EOs have also complex healing properties including antioxidant (Miguel 2010; Serrano et al. 2011), anti-inflammatory (Miguel 2010), immune modulatory (Sadlon and Lamson 2010), and regenerative effect (Woolard et al. 2007). Moreover, combinations of EOs and antimycotics provide further potential in overcoming fungal resistance and in reducing toxicity of both antimycotics and EOs toward mammalian cells.

9.2 Methods of Studying the Combinations Between Essential Oils or Their Components and Antimycotics

Common methods used for study of antimicrobial interactions are disk diffusion, checkerboard and time-kill curves (Verma 2007). All these methods are widely used for both studies on antibiotic and antimycotic combinations. However, they are optimized for antimicrobial substances which are generally hydrophilic in nature. In contrast, EOs are volatile, water insoluble, viscous, and complex substances. Because of this, many researchers proposed different approaches to adjust standard sensitivity and synergy testing methods to the use of EOs.

Special methods are proposed in order to increase water solubility of EOs and to provide better diffusion to the medium. It is achieved by using different solvents, such as DMSO in the concentration not exceeding 1 % v/v (Ahmad et al. 2010; Tangarife-Castaño et al. 2011), DMSO not exceeding 2 % v/v (Silva et al. 2011), DMSO 5 % (Hendry et al. 2009), DMSO 10 % (Amber et al. 2010) or 5 % Tween 80 (Nozaki et al. 2010). At the same time, volatile character of EOs is used by some researchers in assessment of antimicrobial activity produced just by volatile oil phase (Tyagi and Malik 2010; Rózalska et al. 2011).

Disk diffusion method has the easiest performance and because of this can be applied for the screening of large amount of combinations. However, in the studies with EOs this method has serious limitations as evaporation of EOs may significantly distort results. Furthermore, diffusion rate into a medium also significantly differs in various EOs. Difficulties in comparison between results of the studies also take place due to use of different volumes and different dilutions of EOs or their components spotted on disks. Such as, Nozaki et al. (2010) placed on disks 10 μl of EOs or their constituents at the concentration 100 mg/ml when used against *C. albicans* and 200 mg/ml when used against *C. tropicalis*. Ahmad et al. (2010) used eugenol and methyleugenol at the final concentration on disks 500 and 350 μg, respectively. Results in both studies were assessed also in different way. Nozaki et al. (2010) compared diameters of inhibition zones of agents alone and in combination; increase in the diameter of combination over at least one of diameters alone the authors considered as beneficial effect. In turn, Ahmad et al. (2010) and Amber et al. (2010) calculated the sensitivity index (SI) as a ratio between the diameter of inhibition zone (mm) and concentration of drug (mg/ml), which was

also defined as 'clearing' (mm/mg); then the authors compared SIs of agents alone and in combinations.

In the study of combinations between EOs or their components and antimycotics, disk diffusion method is used not widely and only as preliminary assessment (Table 9.1). More common are microdilution and especially checkerboard methods. In the microdilution method there is also no agreement between methodologies in different studies.

There are two main modifications of determining interactions by the dilution method. First modification implies preparation of series dilutions of the first agent (antimycotic) and addition of some definite sub-inhibitory concentration of the EO or its component into the medium with antimycotic dilutions. Then MIC of antimycotic in the presence of EO is compared with MIC of antimycotic alone. Second modification includes preparation of oil/antimycotic mixture in some ratio and then comparison of MICs of mixture with MICs of agents alone. In antimycotic/EO combinations the first modification became more popular (Giordani et al. 2004; Nozaki et al. 2010), while the second one is used more in the study of EOs blends (e.g., Fu et al. 2007). Giordani et al. (2004, 2006) determined MIC 80 % for amphotericin B by a macrobroth dilution method followed by a modelling of fungal growth, and studied its activity either alone or in the presence of different concentrations of *Thymus vulgaris* thymol chemotype EO (0.00031–0.03 μg/ml) (Giordani et al. 2004) and *Cinnamomum cassia* EO (0.0125–0.3 μl/ml) (Giordani et al. 2006). Nozaki et al. (2010) added EOs or their components at concentrations corresponding ½ and ¼ of MIC to media with serial dilutions of amphotericin B. Then the authors determined EOs and their components which caused decreasing MIC of amphotericin B twice, and also oils, which caused decreasing by 4 times or more, such effect actually corresponded to synergism.

Checkerboard method is the most precise and the most commonly used in the studies of combinations between antimycotics and EOs or their components. The majority of studies use the similar procedures, either preparation of stock solutions of both agents and then mixing them at different ratios (Van Vuuren et al. 2009; Van Zyla et al. 2010), or preparation of serial dilutions of both agents and then their paired mixing (Hendry et al. 2009; Amber et al. 2010; Ahmad et al. 2010; Mahboubi and Ghazian Bidgoli 2010; Silva et al. 2011; Tangarife-Castaño et al. 2011). MICs are determined for combinations of all concentrations and then are used for the calculation of fractional inhibitory concentration (FIC) indexes (Verma 2007). Minor discrepancies between studies present in interpretation of FIC indexes. While majority of works uses definition of synergy as FIC index ≤ 0.5, antagonism as FIC index ≥ 4, and indifference for other values (more than 0.5 but less then 4), in some studies another interpretation is present. For example, Van Vuuren et al. (2009) and Van Zyla et al. (2010) defined FIC < 1 as synergy, FIC > 1 as antagonism and FIC $= 1$ as indifference. Mahboubi and Ghazian Bidgoli (2010) defined synergy as FIC ≤ 0.5, antagonism as FIC > 2 and indifference between these values; however, because studied combinations exhibited only FIC indexes less then 0.5, interpretation of results is not changed depending on chosen criteria.

Table 9.1 Methods used in the study of interactions between essential oils or their components and antimycotics

Method	Principle	Advantages	Disadvantages	References
Disk diffusion method	Sterile paper disks are impregnated with a mixture of antimycotic and essential oil and placed onto inoculated medium. After incubation diameters of inhibition zones are measured and compared with diameters for agents used alone. Another modification assumes evaluation of changes in shape of inhibition zone of disk with two agents placed closely to each other.	1. Easy performance allows screening large amount of combinations in short period. 2. It is possible to utilize commercially available disks with antimycotics which increases accuracy in impregnation of the disk with antimycotic.	1. Evaporation of the essential oil from the disk may significantly distort results. 2. Diffusion of essential oils into the medium varies in different essential oils which may also significantly change results. 2. There are no criteria to differentiate between synergistic and indifferent effect. 3. Bacteriostatic and bactericidial effects are not distinguished. 4. Time-dependent interactions are not studied.	Nozaki et al. (2010), Ahmad et al. (2010), Amber et al. (2010)
Dilution methods (agar and broth dilution)	Series of dilutions of antimycotic are prepared, and definite sub-inhibitory concentration of EO is added. After an incubation, MIC of antimycotic in the presence of sub-inhibitory oil concentration is determined and compared with MIC of antimycotic alone. Subcultivations from liquid media tubes without visible growth to a fresh medium allow determining also MBC.	1. Bacteriostatic and bactericidal effects are assessed. 2. Study of blends composed of different ratios of agents gives preliminary information on concentration-dependent interactions. 3. Agar dilution modification allows testing combination on the same medium against several strains simultaneously. 4. Microdilution method allows studying small amount of substance which is important in the work with essential oils and their components.	Time-dependent interactions are not studied.	Giordani et al. (2004), (2006), Nozaki et al. (2010)

(continued)

Table 9.1 (continued)

Method	Principle	Advantages	Disadvantages	References
Checkerboard	Series of dilutions of antimycotic and EO are prepared and mixed by pairs. MICs of combination of each concentrations are determined and are used for the calculation of FIC indexes.	1. Concentration-dependent interactions between agents are assessed. 2. Synergistic, indifferent and antagonistic effects are strictly defined.	1. Method is rather cumbersome and laborious. 2. Calculation of FIC indexes assumes a linear doze–response dependence. 3. Time-dependent interactions are not assessed.	Van Vuuren et al. (2009), Hendry et al. (2009), Ahmad et al. (2010), Van Zyla et al. (2010), Saad et al. (2010), Amber et al. (2010), Mahboubi and Ghazian Bidgoli, (2010), Silva et al. (2011), Tangarife-Castaño et al. (2011)
Time-kill curves	Strain is incubated in liquid media containing studied concentration of agents alone and in combinations; then cell count is performed after definite time intervals.	The most precise method to study time-dependent effect of combination.	The most cumbersome and laborious technique which practically allows studying limit number of combinations.	Nozaki et al. (2010)

MIC Minimal inhibitory concentration, MBC minimal bactericidal concentration, FIC Fractional inhibitory concentration

Classical procedure of time-kill curves assumes making sub-cultures from tubes containing antimicrobial agents and growing microorganisms on a fresh solid medium with calculation of colony-forming units. Although this method gives an opportunity to study time-dependent interactions between agents in combinations, but it is the most cumbersome and laborious. Measuring optical density of liquid cultures in dynamics also gives some information on time-dependent effect of the combination, and such method is used in some studies. One of such studies was performed by Nozaki et al. (2010), who measured optical densities of *C. albicans* cultures in media containing different concentrations of clove oil and amphotericin B at 530 nm in dynamics during 48 h. Although measuring optical density does not allow determining viable cell count, it gives information about time-dependent stability of antimicrobial effect.

9.3 Combinations Between Essential Oils and Antimycotics

9.3.1 Combinations Between Essential Oils and Polyenes

Combinations between antimicrobial agents open wide perspectives in increase of antimicrobial properties and reduction of toxic effect for both combined components (Verma 2007). In contrast to combinations of antibiotics against bacteria, antifungal combinations, especially combinations between synthetic antifungals and natural products, are not so well investigated (Table 9.2).

Giordani et al. (2004) revealed interesting concentration-dependent interactions between *T. vulgaris* EO of thymol chemotype and amphotericin B: higher concentration of oil in the medium (0.01–0.3 μg/ml) had enhancing effect on activity of amphotericin B, with the highest enhancing effect at thyme oil concentration of 0.2 μg/ml (MIC 80 % of amphotericin B decreased by 48 %); while lower oil concentration (0.00031–0.0025 μg/ml) demonstrated antagonistic effect. Two years later, Giordani et al. (2006) published results of the study on combination between *C. cassia* EO and amphotericin B against *C. albicans*. Similar methodology was used: the authors investigated influence of the presence of different concentrations of *C. cassia* EO in the medium on MIC of amphotericin B. EO concentrations 0.08–0.1 μl/ml in the medium caused a decrease of the MIC 80 % of amphotericin B. The characteristic feature of this study is that the authors not only determined effects of agents experimentally but also used mathematical modelling of fungal growth, which helped to determine the most efficient EO concentration in combination with amphotericin B. EO in the concentration of 0.1 μl/ml caused the strongest decrease (by 70 %) of MIC 80 % of amphotericin B. In contrast to thyme oil, no antagonistic effect was detected. The authors emphasized that the potentiation of amphotericin B demonstrated in vitro have potential in the development of less toxic and more effective therapies, which may be especially useful for the treatment of candidiasis in HIV infection.

Table 9.2 Studies on combinations between EOs and antimycotics against *Candida* spp

Essential oils	Antimycotic	Method	Test fungus	Effect	References
Combinations between polyenes and EOs or their components					
Thymus vulgaris	Amphotericin B	Microdilution	*C. albicans*	Concentration of EO 0.01–0.3 μg/ml showed synergistic effect while concentrations 0.00031–0.0025 μg/ml were antagonistic	Giordani et al. (2004)
Cinnamomum cassia	Amphotericin B	Microdilution	*C. albicans*	Enhancing activity of amphotericin B in the presence of studied EO	Giordani et al. (2006)
Origanum vulgare, Pelargonium graveolens, Melaleuca alternifolia	Nystatin	Checkerboard, disk diffusion	*C. albicans, C. krusei, C. tropicalis*	*O. vulgare* showed synergistic effect, *M. alternifolia*—additive effect, with *P. graveolens* effect was indifferent	Rosato et al. (2009)
M. alternifolia, T. vulgaris, Mentha piperita, Rosmarinus officinalis	Amphotericin B	Checkerboard	*C. albicans*	Interactions were mainly antagonistic	Van Vuuren at el. (2009)
Carvacrol	Amphotericin B	Checkerboard	*C. albicans*	Effect was synergistic	Van Zyla et al. (2010)
26 EOs (two bergamot oils, chamomile German, chamomile Roman, clary sage, clove, cypress, two eucalyptus oils, frankincense, juniper, two lavender oils, lemon, lemongrass, melissa, mirrh, neroli, orange, peppermint, rose, rose absolute, rosemary, rose otto, sandalwood, and vetiver) and 11 EO components (1,8-cyneole, citral, citronellal, citronellol, eugenol, farnesol, geraniol, guaiacol, (+)-limonene, linalool, and (-)-α-pinene)	Amphotericin B	Disk diffusion, microdilution, time-kill curves	*C. albicans, C. tropicalis*	Synergy with clove oil against all tested strains, against *C. tropicalis* synergy with amphotericin B was shown by lemongrass and rose otto oils.	Nozaki et al. (2010)
Among EO components synergy was present in eugenol and guaiacol against *C. albicans*, in isoeugenol and *β*-caryophyllene against *C. tropicalis*					
Thymus broussonetii, T. maroccanus	Amphotericin B	Checkerboard	*C. albicans*	Synergy for all combinations	Saad et al. (2010)
Myrtus communis	Amphotericin B	Checkerboard	*C. albicans*	Synergy	Mahboubi and Ghazian Bidgoli (2010)
Coriandrum sativum	Amphotericin B	Checkerboard	*C. albicans, C. tropicalis*	Synergy against *C. albicans* and additive effect against *C. tropicalis*	Silva et al. (2011)
Piper bredemeyeri	Amphotericin B	Checkerboard	*C. albicans*	Indifference	Tangarife-Castaño et al. (2011)

(continued)

Table 9.2 (continued)

Essential oils	Antimycotic	Method	Test fungus	Effect	References
Combinations between azoles and EOs or their components					
T. broussonetii, T. maroccanus	Fluconazole	Checkerboard	*C. albicans*	Synergy for all combinations	Saad et al. (2010)
Ocimum sanctum	Fluconazole, ketoconazole	Checkerboard, disk diffusion	*C. albicans, C. tropicalis, C. parapsilosis, C. glabrata,* including 74 fluconazole-sensitive and 16 fluconazole-resistant isolates	Synergy for all combinations against majority of isolates	Amber et al. (2010)
Eugenol and methyleugenol	Fluconazole	Checkerboard, disk diffusion	*C. albicans, C. tropicalis, C. parapsilosis, C. krusei, C. glabrata*	Interactions were mainly synergistic. No antagonism was observed	Ahmad et al. (2010)
Geranium oil, clove oil, cytronelal	Fluconazole, voriconazole	Modified gradient-diffusion	*C. albicans, C. glabrata*	Synergy between all combinations	Rózalska et al. (2011)
Cinnamaldehyde, citral, eugenol and geraniol	Fluconazole	Checkerboard	*C. albicans* biofilms	The highest synergy was noticed in combination between eugenol and fluconazole	Khan and Ahmad (2012)
Piper bredemeyeri	Itraconazole	Checkerboard	*C. albicans*	Synergy	Tangarife-Castaño et al. (2011)
Combinations between other antimicrobial agents and EOs					
Eucalyptus oil, 1,8-cineole	Chlorhexidine digluconate	Checkerboard	*C. albicans*	Combination with eucalyptus oil was synergistic against both suspension and biofilm cells; combination with 1,8-cineole was synergistic against suspension cells and indifferent against biofilms	Hendry et al. (2009)
M. alternifolia	Silver ions	Checkerboard	*C. albicans*	Indifferent effect	Low et al. (2011)

Nystatin is the antifungal drug for which the use is limited due to side effects, first of all, renal failure. However, its combined application with other agents leading to decrease of dosages may be good option in order to restore its usage. For this purpose Rosato et al. (2009) studied combinations of nystatin with three EOs, *Origanum vulgare, Pelargonium graveolens* and *Melaleuca alternifolia*, against several *Candida* strains. The strongest synergistic effect was present in *O. vulgare* EO with FIC indexes in range of 0.11–0.17. Combinations between nystatin and *P. graveolens* EO showed synergistic effect against some of strains, while combination between nystatin and *M. alternifolia* was only additive.

Van Vuuren et al. (2009) investigated interactions between amphotericin B and EOs of *M. alternifolia*, *T. vulgaris*, *Mentha piperita,* and *Rosmarinus officinalis* against *C. albicans* using checkerboard method. Combinations were studied in nine different ratios (9:1; 8:2; 7:3; 6:4; 5:5; 4:6; 3:7; 2:8, and 1:9) and assessment of results was done by the calculation of FIC indexes, where FIC < 1 was defined as synergy, FIC > 1—antagonism and FIC $= 1$—indifference. Accordingly to these criteria, synergistic interactions were present between *R. officinalis* EO and amphotericin B in ratio 9:1, between *M. alternifolia* EO and amphotericin B in ratios 6:1 and 9:1, and between *M. piperita* EO and amphotericin B also in ratio 9:1. In other ratios, combinations were either indifferent or mainly antagonistic; particularly, in case of *T. vulgaris* EO, all combinations were antagonistic. However, it is worth to emphasize that interpretation of FIC indexes in this study significantly differs from commonly used, such as definition of synergy as FIC index ≤ 0.5, antagonism as FIC index > 4, and indifferent interaction at FIC indexes from 0.5 to 4 (Verma 2007). In the case of application of commonly used criteria, all combinations were indifferent: FIC indexes for the combination between *R. officinalis* EO and amphotericin B were in range from 0.93 to 2.53 depending on ratios of agents, FIC indexes for *M. alternifolia* EO/amphotericin B combination were in range of 0.93–1.73, for *T. vulgaris* EO/amphotericin B—in range of 1.39–2.60, for *M. piperita* EO/amphotericin B—in range from 0.92 to 1.87.

Van Zyla et al. (2010) apart from examination of antimicrobial activity of combinations of several components of EOs, also studied combined effect of carvacrol with amphotericin B against *C. albicans*. The combination showed strong synergy with FIC index of 0.41 achieving MIC values 0.00141 mM for amphotericin B and lower than 1.66 mM for carvacrol. Synergistic effect was explained by the authors due to damaging activity of both agents on fungal cell membrane. Amphotericin B binds selectively to ergosterol with formation of transmembrane pores leading to leakage of intracellular ions and macromolecules with cell death. This, in turn, is enhanced by carvacrol which has non-selective tropism to fungal and bacterial cell membranes.

Nozaki et al. (2010) studied activity of 26 different EOs (two bergamot oils, chamomile German, chamomile Roman, clary sage, clove, cypress, two eucalyptus oils, frankincense, juniper, two lavender oils, lemon, lemongrass, melissa, mirrh, neroli, orange, peppermint, rose, rose absolute, rosemary, rose otto, sandalwood, and vetiver) and 11 EO components (1,8-cyneole, citral, citronellal, citronellol, eugenol, farnesol, geraniol, guaiacol, (+)-limonene, linalool, and (-)-α-pinene)

against two isolates of *C. albicans* and one isolate of *C. tropicalis* by the disk diffusion methods, alone and in combinations with amphotericin B. 10 EOs and 9 EO components were then studied by microdilution method. Synergistic effect was documented in clove EO against all tested strains: MIC of amphotericin B decreased from 0.5-1 μg/ml alone to 0.16 μg/ml in the presence of ½ MIC of clove oil against *C. albicans* and from 4 to 0.25 μg/ml against *C. tropicalis*. Bergamot and chamomile German EOs had additive effect against one of the strains of *C. albicans*. Lemograss and rose otto EOs showed synergy against *C. tropicalis* (MIC of amphotericin B decreased from 4 to 0.5 μg/ml). Furthermore, the majority of studied EO components demonstrated either synergistic or additive effect in combination with Amphotericin B. Eugenol and guaiacol were synergistic against *C. albicans*, while citronellal, citronellol, farnesol, geraniol, and linalool were additive. Against *C. tropicalis* effect was only additive in eugenol, farnesol, and guaiacol. Further advanced investigations revealed that against *C. tropicalis* synergistic effect was present not only in clove oil but in related compounds such as isoeugenol and *β*-caryophyllene.

Saad et al. (2010) investigated combinations between EOs of the two Moroccan endemic thymes, *T. maroccanus* and *T. broussonetii*, and two antifungals, amphotericin B and fluconazole, against *C. albicans*. The FIC indexes of *T. maroccanus* and *T. broussonetii* EOs combined with amphotericin B and fluconazole were calculated from the checkerboard assay and were 0.49, 0.27, 0.37, and 0.3, respectively, and indicated presence of synergy in all combinations. Moreover, fluconazole possessed stronger synergistic effect with EOs compared with amphotericin B.

Mahboubi and Ghazian Bidgoli (2010) studied activity of myrtle EO (*Myrtus communis*) against nine strains of *C. albicans* and different species of *Aspergillus*, and evaluated its combination with amphotericin B. The major active components of the studied oil were 1,8-cineole (36.1 %), alpha-pinene (22.5 %), linalool (8.4 %), bornyl acetate (5.2 %), alpha-terpineol (4.4 %), linalyl acetate (4.2 %), and limonene (3.8 %). Myrtle oil not only exhibited good antifungal activity but also high activity in the combination with amphotericin B, and therefore, has potential in reducing dosages of amphotericin B and its toxic effect. Marked synergy was noticed with FIC index of 0.28 against *C. albicans* and 0.26 against *Aspergillus niger*.

Silva et al. (2011) studied interactions between *Coriandrum sativum* and amphotericin B against three reference strains, *C. albicans* ATCC 90028, *C. albicans* ATCC 24433, and *C. tropicalis* ATCC 750 by checkerboard method. The main component of *C. sativum* EO was linalool (64.38 %); the other major components (>4 %) were α-pinene, p-cymene, camphor, and geranyl acetate. Against both strains of *C. albicans* the combination was synergistic (FIC indexes were 0.375 and 0.185), while against *C. tropicalis* effect was additive with FIC index of 1.0. The authors explained observed results by the inhibition of germ tubes formation in *C. albicans* strains leading to the synergistic effect with amphotericin B related to the permeation of cellular membrane and leakage of intracellular components. In *C. albicans* amphotericin B has effect on germ tube

formation potentiated by coriander oil. At the same time, *C. tropicalis* is characterized by inability to produce germ tubes and, therefore, coriander oil cannot potentiate effect of amphotericin B against this species.

Tangarife-Castaño et al. (2011) studied anti-candida activity against reference and clinical strains of 59 plant EOs and interactions of the most active of them, *Piper bredemeyeri* EO, with amphotericin B and itraconazole by checkerboard method. Combination with amphotericin B showed indifference with FIC index of 1.06, while with itraconazole it was strongly synergistic (FIC index was in ranges 0.09–0.13). The authors also demonstrated inhibition of germ tube formation by *P. bredemeyeri* EO and suggested its action on an important process in the morphotransformation of *C. albicans* from yeast to filamentous form, along with the affecting membrane integrity or reorganization of the fungal cytoskeleton. The major components of the oil were monoterpene hydrocarbons alpha- and beta-pinene (20.3 and 32.3 %, respectively), to which such antifungal action was attributed. Alpha- and beta-pinene have shown to destroy cellular integrity in fungi; they inhibit respiration and transportation of ions, and also they can increase membrane permeability in *C. albicans* (Tangarife-Castaño et al. 2011).

9.3.2 Combinations Between Essential Oils and Azole Antimycotics

The EO of *Ocimum sanctum* and its combination with two azoles, fluconazole, and ketoconazole, against both fluconazole-sensitive and resistant isolates was studied by Amber et al. (2010). The major component of *O. sanctum* EO was identified as methyl chavicol, linalool, limonene, carvone, and alpha-caryophyllene, among them methyl chavicol and linalool were found to be the most active constitutes with MIC values ranging from 125 to 200 μg/ml and from 200 to 250 μg/ml, respectively. *O. sanctum* EO was active also against intrinsically resistant to fluconazole isolates. Moreover, its combination with both fluconazole and ketoconazole showed synergistic effect against majority isolates with FIC indexes in ranges from 0.24 to 0.53 and from 0.25 to 0.54, respectively.

Ahmad et al. (2010) tested 64 fluconazole-susceptible and 34 fluconazole-resistant *Candida* strains studying the activity of two EO components, eugenol, and methyleugenol, alone and in combination with fluconazole. Eugenol and methyleugenol are components of EOs with documented high antimicrobial activity, such as clove (*Eugenia caryophyllus*) and basil (*O. sanctum*) EOs. Among susceptible isolates combination between eugenol and fluconazole exhibited indifferent effect against six strains, while against others effect was synergistic; combination between methyleugenol and fluconazole had indifferent effect in five cases, in other 59—synergistic. Among resistant isolates eugenol/fluconazole combination was indifferent in five cases and in other 29—synergistic, methyleugenol/fluconazole combination was indifferent in three cases and in other—synergistic. Therefore, both eugenol and methyleugenol showed great potency in

enhancement of fluconazole activity against resistant isolates. Mode of action of eugenol and methyleugenol is not much studied and mostly is attributed to entering between the fatty acid chains of fungal membrane bilayers, altering their fluidity and permeability. Damage to cell membranes disregulates important membrane-bound enzymes that catalyze the synthesis of a number of major polysaccharide components of cell wall, including β-glucans, chitin, and mannan. This, in turn, disturbs cell growth and envelope morphogenesis (Ahmad et al. 2010). Mechanism of action of fluconazole, like in other azoles, is in inhibiting the fungal cytochrome P-450-dependent enzyme lanosterol 14-α-demethylase which converts lanosterol to ergosterol. Inhibition of this enzyme disrupts synthesis of fungal membrane (Sheehan et al. 1999; Pfaller et al. 2006). Synergistic effect between eugenol or methyleugenol and fluconazole can be explained by simultaneous damage to fungal membranes.

Rózalska et al. (2011) proposed a modified gradient-diffusion method of MIC Test Strip for assessment of EO-antifungal combinations in the liquid and volatile phases, and studied action of combinations between geranium, clove oils, cytronelal, and two antifungals, fluconazol and voriconazole, against *C. albicans* and *C. glabrata*. Geranium oil applied in concentration of ½ MIC caused decrease in fluconazole MIC from 12.0 μg/ml to 0.064 μg/ml and voriconazole MIC from 0.125 μg/ml to 0.006 μg/ml against *C. albicans* in agar dilution test. The similar effect was observed against *C. glabrata*, and also by using volatile clove oil and cytronelal in sub-inhibitory MIC.

Study involving fungal biofilms was performed by Khan and Ahmad (2012). Two strains of *C. albicans*, reference and clinical, were used for production of biofilms and the effect of four EO components, cinnamaldehyde, citral, eugenol, and geraniol, either alone or in combination with fluconazole was studied. Inhibitory effect of the test compounds on fungal biofilms was determined by the XTT reduction assay, light microscopy, and scanning electron microscopy (SEM); while effect of combinations was assessed using checkerboard method. The highest inhibitory effect was observed in eugenol and cinnamaldehyde, which was higher than in the two other compounds and also higher than in fluconazole and amphotericin B. SEM showed that target of tested compounds in both biofilm and planktonic cells was the cell membrane. The highest synergy was revealed in eugenol/fluconazole combination against reference *C. albicans* strain, as proved by FIC index of 0.14.

9.4 Combinations Between Essential Oils and Other Antimicrobial Agents Against *Candida* spp

9.4.1 Combinations Between Essential Oils and Antiseptics Against Candida spp

Hendry et al. (2009) studied combinations made of eucalyptus EO or its major component 1,8-cineole and antiseptic chlorhexidine digluconate against several

bacteria and *C. albicans* by checkerboard method. The study is especially interesting because combinations were tested against planktonic and biofilm cells of microorganisms. Combination between crude eucalyptus oil and chlorhexidine digluconate exhibited synergy against both suspension and biofilm cells of *C. albicans*; furthermore, synergy was especially evident in biofilm form (FIC index was 0.094, while for suspension cells FIC index was 0.500). Combination between 1,8-cineole and chlorhexidine digluconate was synergistic against cells in suspension (FIC index 0.469) but indifferent against biofilm cells (FIC index 1.125). From these results the authors made a conclusion that the combination between crude eucalyptus EO and chlorhexidine digluconate is preferable in order to inhibit the growth of *C. albicans* compared with pure 1,8-cineole.

Synergistic effect between either eucalyptus oil or its component 1,8-cineole with chlorhexidine digluconate was explained by attacking the same target in fungal cell—cytoplasmic membrane, which resulted in damaging the structural stability of fungal cell and increasing membrane permeability.

9.4.2 Combinations Between Essential Oils and Silver Ions Against Candida spp

Silver in form of silver ions and silver nanoparticles is broadly investigated and used to inhibit growth of different microorganisms including fungi (Zhang et al. 2006; Monteiro et al. 2011; Kumar and Mamidyala 2011). Combining silver nanoparticles with other agents possessing antifungal activity has potential in decreasing their toxicity, as well as in enhancing antifungal effect. However, studies on combinations of silver nanoparticles or ions with antifungals are very limited and mainly devoted to traditional antimycotics, for example, to combination with fluconazole (Gajbhiye et al. 2009).

A study on combination between silver ions and EO of *M. alternifolia* was performed by Low et al. (2011), who investigated activity of tea tree oil in combination with silver ions against three microbial species, two bacterial strains—*Pseudomonas aeruginosa* and *Staphylococcus aureus*, and one *C. albicans* strain. The assessment of results was done by determination of fractional lethal concentration index (FLCI). Combination was in general active but effect on fungi was weaker than on bacteria. Action of combination decreased in the following order *P. aeruginosa* > *S. aureus* > *C. albicans* with FLCI 0.263, 0.663, and 1.197, respectively. However, in spite of the absence of synergistic effect against *C. albicans*, combination between tea tree oil and silver ions can be anyway used as a base for decreasing doses of both agents and, therefore, potential in decreasing side effects.

9.5 Conclusions and Future Perspectives

Combinations between EOs or their components and antimycotics have shown promising activity against both antimycotic-sensitive and resistant *Candida* isolates. The best studied are combinations composed of EOs and either amphotericin B or fluconazole. There are studies devoted either to crude EOs or to discrete components. However, in general, in contrast to antibiotic or antimycotic combinations, combinations between antimycotics and natural products are studied insufficiently and require more attention.

Ongoing studies on antimycotic-EOs interactions should be devoted to better understanding the mechanisms of synergistic and antagonistic effects, which can be done by evaluation of combinations composed of antimycotics and discrete EO components with observation of visible and metabolic changes in fungal cells.

Reviewing methods used for the studies of combinations showed absence of uniform standard techniques which makes it more difficult to compare results obtained in different studies. Further efforts, therefore, should be directed to standardization of techniques and criteria of scientific researches used in studies of combinations composed of natural products.

Most *Candida* infections belong to topical infections for which application of EOs is not difficult. However, pharmaceutical formulations applicable for systemic use of combinations composed of antimycotics and EOs or their components should be developed and evaluated in vitro and in vivo. Future studies should be also directed to combining EOs not only with antimycotics but with other agents possessing antifungal activity, such as metallic nanoparticles and quorum-sensing inhibitors.

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References

Ahmad A, Khan A, Khan LA, Manzoor N (2010) In vitro synergy of eugenol and methyleugenol with fluconazole against clinical *Candida* isolates. J Med Microbiol 59(Pt 10):1178–1184

Amber K, Aijaz A, Immaculata X, Luqman KA, Nikhat M (2010) Anticandidal effect of *Ocimum sanctum* essential oil and its synergy with fluconazole and ketoconazole. Phytomedicine 17(12):921–925

Anderson JB (2005) Evolution of antifungal-drug resistance: mechanisms and pathogen fitness. Nat Rev Microbiol 3(7):547–556

Badiee P, Alborzi A (2011) Susceptibility of clinical *Candida* species isolates to antifungal agents by E-test, Southern Iran: A five year study. Iranian J Microbiol 3(4):183–188

Baldauf SL, Roger AJ, Wenk-Siefert I, Doolittle WF (2000) A kingdom-level phylogeny of eukaryotes based on combined protein data. Science 290(5493):972–977

Bassolé IH, Juliani HR (2012) Essential oils in combination and their antimicrobial properties. Molecules 17(4):3989–4006

Casalinuovo IA, Di Francesco P, Garaci E (2004) Fluconazole resistance in *Candida albicans*: a review of mechanisms. Eur Rev Med Pharmacol Sci 8(2):69–77

Fu Y, Zu Y, Chen L, Shi X, Wang Z, Sun S, Efferth T (2007) Antimicrobial activity of clove and rosemary essential oils alone and in combination. Phytother Res 21(10):989–994

Gajbhiye M, Kesharwani J, Ingle A, Gade A, Rai M (2009) Fungus-mediated synthesis of silver nanoparticles and their activity against pathogenic fungi in combination with fluconazole. Nanomedicine 5(4):382–386

Garozzo A, Timpanaro R, Stivala A, Bisignano G, Castro A (2011) Activity of Melaleuca alternifolia (tea tree) oil on Influenza virus A/PR/8: study on the mechanism of action. Antiviral Res 89(1):83–88

Giordani R, Regli P, Kaloustian J, Mikaïl C, Abou L, Portugal H (2004) Antifungal effect of various essential oils against *Candida albicans*. Potentiation of antifungal action of amphotericin B by essential oil from *Thymus vulgaris*. Phytother Res 18(12):990–995

Giordani R, Regli P, Kaloustian J, Portugal H (2006) Potentiation of antifungal activity of amphotericin B by essential oil from *Cinnamomum cassia*. Phytother Res 20(1):58–61

Dannaoui E, Desnos-Ollivier M, Garcia-Hermoso D, Grenouillet F, Cassaing S, Baixench MT, Bretagne S, Dromer F, Lortholary O and French Mycoses Study Group (2012) Candida spp. with acquired echinocandin resistance, France, 2004–2010. Emerg Infect Dis 18(1):86–90

Hendry ER, Worthington T, Conway BR, Lambert PA (2009) Antimicrobial efficacy of eucalyptus oil and 1,8-cineole alone and in combination with chlorhexidine digluconate against microorganisms grown in planktonic and biofilm cultures. J Antimicrob Chemother 64(6):1219–1225

Huang M, Kao KC (2012) Population dynamics and the evolution of antifungal drug resistance in *Candida albicans*. FEMS Microbiol Lett 333(2):85–93

Hyldgaard M, Mygind T, Meyer RL (2012) Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Front Microbiol 3:12

Kelly SL, Lamb DC, Corran AJ, Baldwin BC, Kelly DE (1995) Mode of action and resistance to azole antifungals associated with the formation of 14 alpha-methylergosta-8,24(28)-dien-3 beta,6 alpha-diol. Biochem Biophys Res Commun 207:910–915

Khan MS, Ahmad I (2012) Antibiofilm activity of certain phytocompounds and their synergy with fluconazole against *Candida albicans* biofilms. J Antimicrob Chemother 67(3):618–612

Krcmery V, Barnes AJ (2002) Non-albicans *Candida* spp. causing fungaemia: pathogenicity and antifungal resistance. J Hosp Infect 50:243–260

Kumar CG, Mamidyala SK (2011) Extracellular synthesis of silver nanoparticles using culture supernatant of *Pseudomonas aeruginosa*. Colloids Surf B Biointerfaces 84(2):462–466

Low WL, Martin C, Hill DJ, Kenward MA (2011) Antimicrobial efficacy of silver ions in combination with tea tree oil against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. Int J Antimicrob Ag 37(2):162–165

Mahboubi M, Ghazian Bidgoli F (2010) In vitro synergistic efficacy of combination of amphotericin B with *Myrtus communis* essential oil against clinical isolates of *Candida albicans*. Phytomedicine 17(10):771–774

Martins MD, Lozano-Chiu M, Rex JH (1997) Point prevalence of oropharyngeal carriage of fluconazole-resistant *Candida* in human immunodeficiency virus-infected patients. Clin Infect Dis 25:843–846

Martins MD, Rex JH (1996) Resistance to antifungal agents in the critical care setting: problems and perspectives. New Horiz 4:338–344

Matsumori N, Yamaji N, Matsuoka S, Oishi T, Murata M (2002) Amphotericin B covalent dimers forming sterol-dependent ion-permeable membrane channels. J Amer Chem Soc 124:4180–4181

Miguel MG (2010) Antioxidant and anti-inflammatory activities of essential oils: a short review. Molecules 15(12):9252–9287

Monteiro DR, Gorup LF, Silva S, Negri M, de Camargo ER, Oliveira R, Barbosa DB, Henriques M (2011) Silver colloidal nanoparticles: antifungal effect against adhered cells and biofilms of *Candida albicans* and *Candida glabrata*. Biofouling 27(7):711–719

Monzote L, García M, Montalvo AM, Scull R, Miranda M, Abreu J (2007) In vitro activity of an essential oil against Leishmania donovani. Phytother Res 21(11):1055–1058

Nozaki A, Takahashi E, Okamoto K, Ito H, Hatano T (2010) Antifungal activity of essential oils and their constituents against *Candida* spp. and their effects on activity of amphotericin B. Yakugaku Zasshi 130(6):895–902

Palmeira-de-Oliveira A, Salgueiro L, Palmeira-de-Oliveira R, Martinez-de-Oliveira J, Pina-Vaz C, Queiroz JA, Rodrigues AG (2009) Anti-*Candida* activity of essential oils. Mini Rev Med Chem. 9(11):1292–1305

Pauli A (2006) Anticandidal low molecular compounds from higher plants with special reference to compounds from essential oils. Med Res Rev 26(2):223–268

Pemán J, Cantón E, Quindós G, Eraso E, Alcoba J, Guinea J, Merino P, Ruiz-Pérez-de-Pipaon MT, Pérez-del-Molino L, Linares-Sicilia MJ, Marco F, García J, Roselló EM, Gómez-G-de-la-Pedrosa E, Borrell N, Porras A, Yagüe G and FUNGEMYCA Study Group (2012) Epidemiology, species distribution and in vitro antifungal susceptibility of fungaemia in a Spanish multicentre prospective survey. J Antimicrob Chemother 67(5):1181–1187

Pfaller MA (2012) Antifungal drug resistance: mechanisms, epidemiology, and consequences for treatment. Am J Med 125(1 Suppl):S3–S13

Pfaller MA, Diekema DJ, Sheehan DJ (2006) Interpretive breakpoints for fluconazole and *Candida* revisited: a blueprint for the future of antifungal susceptibility testing. Clin Microbiol Rev 19(2):435–447

Pfaller MA, Castanheira M, Lockhart SR, Ahlquist AM, Messer SA, Jones RN (2012) Frequency of decreased susceptibility and resistance to echinocandins among fluconazole-resistant bloodstream isolates of *Candida glabrata*. J Clin Microbiol 50(4):1199–1203

Rai M, Mares D (eds) (2003) Plant-derived antimycotics: current trends and future prospects. Food Products Press, NY

Reichling J, Schnitzler P, Suschke U, Saller R (2009) Essential oils of aromatic plants with antibacterial, antifungal, antiviral, and cytotoxic properties–an overview. Forsch Komplementmed 16(2):79–90

Rosato A, Vitali C, Piarulli M, Mazzotta M, Argentieri MP, Mallamaci R (2009) In vitro synergic efficacy of the combination of nystatin with the essential oils of *Origanum vulgare* and *Pelargonium graveolens* against some *Candida* species. Phytomedicine 16(10):972–975

Rózalska B, Sadowska B, Wieckowska-Szakiel M, Budzyńska A (2011) The synergism of antifungals and essential oils against *Candida* spp. evaluated by a modified gradient-diffusion method. Med Dosw Mikrobiol 63(2):163–169

Saad A, Fadli M, Bouaziz M, Benharref A, Mezrioui NE, Hassani L (2010) Anticandidal activity of the essential oils of *Thymus maroccanus* and *Thymus broussonetii* and their synergism with amphotericin B and fluconazol. Phytomedicine 17(13):1057–1060

Sadlon AE, Lamson DW (2010) Immune-modifying and antimicrobial effects of Eucalyptus oil and simple inhalation devices. Altern Med Rev 15(1):33–47

Sanglard D, Odds FC (2002) Resistance of *Candida* species to antifungal agents: molecular mechanisms and clinical consequences. Lancet Infect Dis 2(2):73–85

Serrano C, Matos O, Teixeira B, Ramos C, Neng N, Nogueira J, Nunes ML, Marques A (2011) Antioxidant and antimicrobial activity of Satureja montana L. extracts. J Sci Food Agric 91(9):1554–1560

Sheehan DJ, Hitchcock CA, Sibley CM (1999) Current and emerging azole antifungal agents. Clin Microbiol Rev 12(1):40–79

Silva F, Ferreira S, Duarte A, Mendonça DI, Domingues FC (2011) Antifungal activity of *Coriandrum sativum* essential oil, its mode of action against *Candida* species and potential synergism with amphotericin B. Phytomedicine 19(1):42–47

Tangarife-Castaño V, Correa-Royero J, Zapata-Londoño B, Durán C, Stanshenko E, Mesa-Arango AC (2011) Anti-*Candida albicans* activity, cytotoxicity and interaction with antifungal drugs of essential oils and extracts from aromatic and medicinal plants. Infectio 15(3):160–167

Tyagi AK, Malik A (2010) Liquid and vapour-phase antifungal activities of selected essential oils against candida albicans: microscopic observations and chemical characterization of cymbopogon citratus. BMC Complement Altern Med 10:65

Van Vuuren SF, Suliman S, Viljoen AM (2009) The antimicrobial activity of four commercial essential oils in combination with conventional antimicrobials. Let Appl Microbiol 48(4):440–446

Van Zyla RL, Seatlholof ST, Van Vuuren SF, Viljoen A (2010) Pharmacological interactions of essential oil constituents on the viability of micro-organisms. Natur Prod Commun 5(9):1381–1386

Verma P (2007) Methods for determining bactericidal activity and antimicrobial interactions: synergy testing, time-kill curves, and population analysis. In: Schwalbe R, Steele-Moore L, Goodwin AC (eds) Antimicrobial susceptibility testing protocols. CRC Press, New York, pp 276–277

White TC, Marr KA, Bowden RA (1998) Clinical, cellular, and molecular factors that contribute to antifungal drug resistance. Clin Microbiol Rev 11:382–402

Woollard AC, Tatham KC, Barker S (2007) The influence of essential oils on the process of wound healing: a review of the current evidence. J Wound Care 16(6):255–257

Zhang S, Ahearn DG, Mateus C and Crow SAJr (2006) In vitro effects of Ag + on planktonic and adhered cells of fluconazole-resistant and susceptible strains of *Candida albicans*, *C. glabrata* and *C. krusei*. Biomaterials 27(13):2755–2760

Chapter 10
Flavonoids as Antifungal Agents

Roseli Maria De Conti Lourenço, Patricia da Silva Melo and Ana Beatriz Albino de Almeida

Abstract Flavonoids are phenolic compounds widely distributed in the plant kingdom. These natural compounds have been known to be active against a variety of microorganisms. This chapter aims to report the efforts of the scientific discoveries and developments of new antifungal flavonoid molecules and also to understand the flavonoids chemistry. An overview of the recent papers on the antifungal activity of flavonoids which represent a potential alternative to conventional fungicides is presented.

10.1 Introduction

Flavonoids constitute the largest group of polyphenols, and are considered to be responsible for the color and taste of many fruits and vegetables. Flavus means yellow in Latin. This group of natural products shows extraordinary diversity and variation. Flavonoids are secondary metabolites which together with other plant compounds share a common origin: the amino acid phenylalanine and the acetate coenzyme A esters. These compounds are generally synthesized by the shikimate pathway, from which they are produced using carbohydrate metabolism. Secondary metabolites are distinct from the components of intermediary metabolism in that they are nonessential for the basic metabolic process of growth and development in plant. In spite of this, they are indeed crucial for many important functional aspects of plant life (Bueno et al. 2012).

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It was suggested that the biochemical and pharmacological activities of phenolic compounds, in particular flavonoids, are due to their long association with animal species and other organisms (Ndhlala et al. 2010). Some of the proven biological activities include antimicrobial, antiviral, antiinflammatory, antiallergic, vasodilator effects and inhibition of lipid peroxidation (Ndhlala et al. 2010), anticancer activity (Cushinie and Lamb 2005; Estelle et al. 2011), enzyme inhibition (Cushinie and Lamb 2005), antiulcer (Batista et al. 2004), antioxidant (Karimi et al. 2011), antidiarrheal, antidiabetic, anti-inflammatory (Cushinie and Lamb 2005), antiplasmodial, antihypertensive, anticonvulsant, antinociceptive (Ojewole et al. 2010), and nutraceutical (Tapas et al. 2008).

Harborne and Williams (2000) extensively reviewed the advances of the flavonoids science since 1975. There are excellent reviews on the flavonoid properties as antimicrobial agents and many other biological activities as well as cytotoxicity (Nijveldt et al. 2010; Cesco et al. 2012; Zheng et al. 2012; Pierson et al. 2012; Wink et al. 2012). Phenolic compounds as flavonoids have a considerable dual mode of action: reducing risks of severe human diseases and promoting a deleterious impact due to adverse side effects. The classical antioxidant capacity of flavonoids might exert modulatory activities in cells through cell signaling acting mainly in protein kinases. These interactions could trigger unpredictable results, depending on cell type, cell cycle, and the kind of stimulus applied (Kyselova 2011).

The flavonoids biosynthesis, differential in situ accumulation of flavonoids in various pathway mutants as well as flavonoid uptake and movement within the plant has been reviewed by Buer et al. (2010). In this work the roles played by flavonoids in plant physiology and development, were discussed including efforts to define molecular targets for flavonoids.

Types and amounts of plant-released flavonoids concerning their effects on soil activities have been studied by Cesco et al. (2012). The effects of root-borne flavonoids on mycorrhizal and pathogenic fungi have been reviewed.

A very recent review has been carried out on polyphenol including flavonoids mainly concerning their analysis and antioxidant capacity as well as their large amount of biological properties concerning human health. Also, standardized methods for the determination of antioxidant capacity and anthocyanin pigments in foods and diets have been published. In this paper various recently published reviews on the biological properties of flavonoids are stated (Bueno et al. 2012).

The occurrence of potential bioactive compounds isolated and identified from a selection of tropical fruit plants of importance in Australia, was reported by Pierson et al. (2012). In this work secondary metabolites isolated from fruits such as flavonoids, flavones, flavonols, isoflavonols, isoflavones, and anthocyanins with biological activities have been extensively discussed.

An up-to-date paper report on the way that botany, ethnobotany, phytochemistry, and pharmacology of extracts from *Rheum australe*, which constituted of many different secondary metabolites including many flavonoid compounds were extensively studied by Rokaya et al. (2012). The antifungal activity of the plant extracts, as well as many of their other biological activities, was extensively reviewed. The

possible uses of *Rheum australe* species to treat different diseases have been highlighted providing a basis for future research.

Phyllantus amaru, a small Indian herb of the Euphorbiaceae family, with medicinal properties used Worldwide, was reviewed by Patel et al. (2011) covering literature from 1980 to 2011. Phytochemical studies of *P. amaru* showed to be constituted of many active compounds including the flavonoid class of natural products. The extracts and the isolated compounds from *P. amarus* showed a wide spectrum of pharmacological activities (including antifungal activity) thus emphasizing the extensive potential of this plant for future work and commercial exploitation.

Himesh et al. (2011) reported on a preliminary screening and HPLC analysis of flavonoids from methanolic extracts of leaves of *Annona squamosa*, commonly known as custard apple, and cultivated throughout India. The data generated in this work have provided the basis for its wide uses as the therapeutics in traditional and folk medicines.

Very important and extensive review papers related to the pharmacological properties of the flavonoids were published showing the important role of this class of compounds for the discovery of new drugs which may possess a wide range of applications (Cowan 1999; Cushnie and Lamb 2005; Friedman 2007).

Structural analysis of flavonoids is of great interest. Pinheiro and Justino (2010) reported their spectroscopic application.

Sophora, a genus of the Fabaceae family is widely distributed in Asia, Oceania, and the Pacific Islands. It is commonly used in traditional Chinese Medicine. It was found to possess various pharmacological and therapeutic properties due to its active components; such as many alkaloids, along with flavonoids, isoflavonoids, isoprenylated flavonoids, isoflavones, flavones, flavonols and its glycosides, coumarochromes, saponins, triterpene glycosides, phospholipids, polysaccharides, and fatty acids (Krishna et al. 2012). In a review paper the authors discussed the ethnopharmacology, the biological activities, and the correlated chemical compounds of genus *Sophora*.

Recently, many reviews concerning the antifungal, antibacterial, or antiviral activities of flavonoids have been published (Recio and Rios 1989; Harborne and Williams 2000; Cushnie and Lamb 2005; Friedman 2007; Orhan et al. 2010; Cesco et al. 2012; Pierson et al. 2012; Zheng et al. 2012)

Tapas et al. (2008) reported the nutraceutical properties of flavonoids showing the important role of the antifungal properties of this class of compounds.

The antifungal properties along with many other biological activities of *Artocopus* species, which is known to occupy a variety of ecological niches across different habitats, also possessing many flavonoid compounds as their components were described (Jagtap and Bapat 2010).

A review by Wink et al. (2012) summarizes the evidence that secondary metabolites from plants such as alkaloids, phenolic compounds (flavonoids, isoflavonoids, and anthocyanins), and terpenoids can interfere with ABC transporters in cancer cells, parasites, bacteria, and fungi. Polar phenolic compounds such as phenolic acids, flavonoids, catechins, chalconnes, xanthones, stilbenes,

anthocyanin, tannins, anthraquinones, and naphthoquinones are shown to directly inhibit proteins to form several hydrogen and ionic bonds and thus to disturb the 3D structure transporters. These natural products may be of interest in medicine or agriculture as they can enhance the activity of chemotherapeutic compounds or pesticides or even reverse multidrug resistance (at least partially) of adapted and resistant cells. These secondary metabolites when applied in combination with a cytotoxic antimicrobial agent, may reverse resistance in a synergistic fashion.

10.2 Chemical Structure of Flavonoids

Flavonoids are low molecular weight bioactive polyphenols (Sandhar et al. 2011). The basic structural feature of flavonoid is 2-phenyl-benzo-γ-pyrane nucleus consisting of two benzene rings (A and B) linked through a heterocyclic pyran ring (C). They can occur both as the free form aglycones and as glycosides, and differ in their substituents (type, number, and position) and in their unsaturation. Figure 10.1 shows the basic structure of the different flavonoid classes.

The most common classes are flavones, flavonols, flavanones, catechins, isoflavones, and anthocyanins, which account for around 80 % of flavonoids. All flavonoids share a basic C6-C3-C6 phenyl-benzopyran backbone. The position of the phenyl ring relative to the benzopyran moiety allows a broad separation of these compounds into flavonoids (2-phenyl-benzopyrans, isoflavonoids (3-phenyl-benzopyrans) and neoflavans 4-phenyl- benzopyrans) (Pinheiro and Justino 2010) (Fig. 10.2).

Flavonoids differ in their arrangement of hydroxyl, methoxyl, and glycoside side groups and in the conjunction between the A and B rings. A variation in the C ring provides a division of subclasses. According to their molecular structure, they are divided into eight classes (Fig. 10.3) (Pinheiro and Justino 2010).

10.3 Medicinal Plants as Therapeutic Agents

The use of higher plants and preparations made from them to treat infections is an age-old practice and possibly it was the only method available. Since time began,

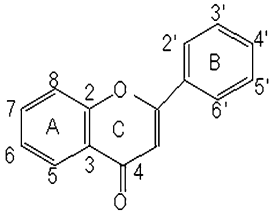


Fig. 10.1 Basic structure of flavonoids

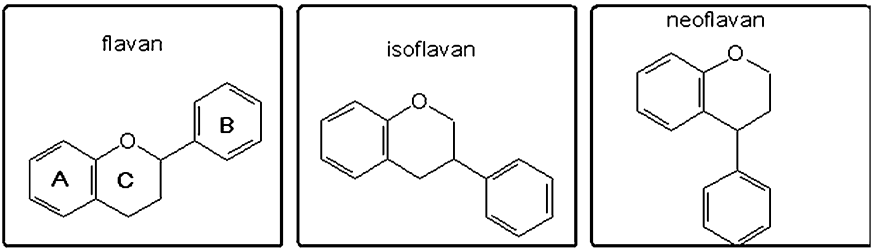


Fig. 10.2 Position of phenyl ring relative to benzopyran

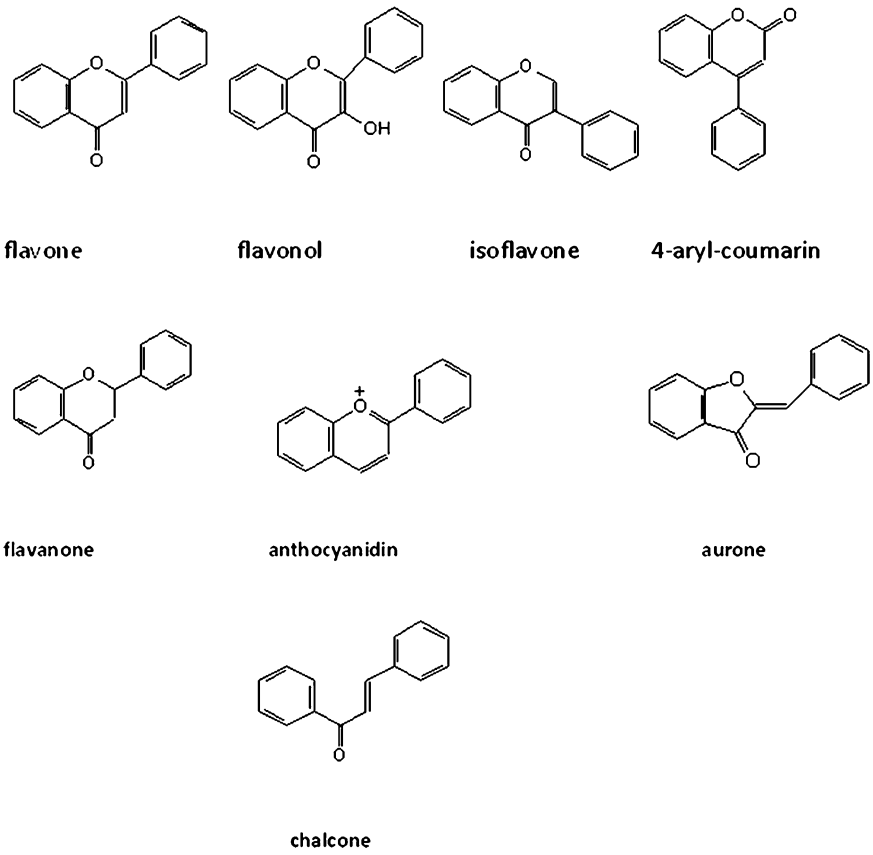


Fig. 10.3 Flavonoid subclasses according to the variation in C Ring

man has used plants to treat common infectious diseases and some of the traditional medicines are still included as part of the habitual treatment of various diseases (Recio and Rios 1989; Rios and Recio 2005). Archeologists have shown

that around 50,000 B.C., humans used plants for flavoring meats and, in ancient Egypt and Assyria, herbs and spices were used for medicinal purposes (Kaefer and Milner 2008). People on all continents applied poultices and drank infusions of hundreds if not thousands, of indigenous plants, dating back to prehistory times (Cowan 1999). Plants are still widely used in ethnomedicine around the world. Historically, therapeutic results have been mixed; and quite often cures or relief symptoms resulted. Poisoning also occurred at a high rate (Cowan 1999). The increase in the use of dietary supplements is related to the concept of "wellbeing," but has been associated with toxic effects. One example is the several cases of aloe-induced hepatotoxicity in contrast to the anti-inflammatory, wound, and immune modulation pharmacologic effects (Yang et al. 2010).

A large body of evidence has accumulated to demonstrate the promising potential of medicinal plants. They are rich in a wide variety of secondary metabolites such as tannins terpenoids, alkaloids, flavonoids ,etc., which have been found to have antimicrobial properties (Cushnie and Lamb 2005). The molecular structures of these secondary metabolites can interfere with group targets in microorganisms such as proteins, nucleic acids, and bio membranes (Wink et al. 2012).

Recently, there has been an increasing interest in the biological properties of flavonoids from medicinal plants which have been used in folk medicine (Recio and Rios 1989; Harborne and Williams 2000; Sohn et al. 2004; Cushnie and Lamb 2005; Kumar et al. 2006; Friedman 2007; Tapas et al. 2008; Sher 2009; Ndhlala et al. 2010; Ojewole et al. 2010; Orhan et al. 2010; Sandhar et al. 2011; Karimi et al. 2011; Kanwal et al. 2011; Ruddock et al. 2011; Bueno et al. 2012; Cesco et al. 2012; Kuete et al. 2012; Rokaya et al. 2012; Zampini et al. 2012; Zheng et al. 2012).

Nowadays scientists have realized an immense potential in natural products from medicinal plants such as flavonoids, which may serve as alternative source of combating infections in human beings with lower cost and lesser toxicity than synthetic compounds (Recio and Rios 1989; Sher 2009; Himesh et al. 2011). This area of research is of particular interest and holds out the possibility of microbial drug development based upon novel structures and new biochemical target enzymes.

10.4 Antifungal Properties of Flavonoids

Flavonoids are a group of about 4,000 naturally occurring compounds known to have contribution to human health through our daily diet; generally they are found as plant pigments in fruit, vegetables, nuts, seeds, stems and flowers as well as tea, wine, propolis, and honey, and represent a common constituent of the human diet (Sharma et al. 2011). In the US, daily dietary intake of mixed flavonoids is estimated to be in the range 500–1,000 mg, but this Fig. can be as high as several grams for people supplementing their diets with flavonoids or flavonoid-containing herbal preparations. The dietary intake of flavonoids is estimated to be 1–2 g/day (Sandhar et al. 2011). The function of flavonoids in flowers is to provide colors

attractive to pollinators and in several other processes, such as pigmentation, response to environmental stresses, plant growth regulation, resistance against pathogens, etc. (Fraga et al. 2010). In leaves, these compounds are increasingly believed to promote the physiological survival of the plant, protecting it from, for example, fungal pathogens and UV-B irradiation. In addition, flavonoids are involved in photosensitization, energy transfer, the actions of plant growth hormones and hormone regulators, control of respiration and photosynthesis, morphogenesis, and sex determination. They are involved in the defense of plants against invading pathogens including insects, bacteria, fungi, and viruses (Cowan 1999; Cushinie and Lamb 2005; Friedman 2007; Sher 2009; Ojewole et al. 2010; Orhan et al. 2010; Kanwal et al. 2011; Ruddock et al. 2011).

The wide range of biological activities of flavonoids is attributed to their capacity to exert effects as antioxidants, free radical scavengers, and chelators of divalent cations. They are also demonstrated to inhibit a variety of enzymes like hydrolases, hyaluronidase, alkaline phosphatase, arylsulfatase, cAMP phosphodiesterase, lipase, α-glucosidase, kinase (Sandhar et al. 2011).

Resistance to antimicrobial agents has become an increasing and pressing global problem (Cushinie and Lamb 2005). Pathways of resistance are complex, including activation of ATP, binding cassette (ABC) transporters, activation of enzymes of cytochrome P-450, and conjunction with glutathione (Wink et al. 2012). Fungal infections, especially ringworm infections and dermatophytosis, can affect various parts of the body, such as the skin, hair, or nails (Ponnusamy et al. 2010; Zheng et al. 2012). The increasing resistance of microorganisms against available antimicrobial agents is of major concern among scientists and clinicians worldwide. The World Health Organization cited that antimicrobial resistance is one of the three most serious problems for public health (Bassetti et al. 2011). To develop new or more efficient and safe antifungal drugs is an urgent requirement (Ahmidi et al. 2010; Zheng et al. 2012). Despite the advances in science and technology, surprisingly the development of novel and efficient antifungal drugs is still lagging behind due to the very fact that fungi are also eukaryotic cells and have mechanisms similar to human beings. Hence it becomes very difficult to develop an antifungal agent that is more specific in targeting the fungi alone without any damage to human beings (Lakshmipathy and Kannabiran 2010). To overcome the drawbacks of the current antimicrobial drugs and to obtain more efficient drugs, an antimicrobial drug having a novel mode of action should be found (Orhan et al. 2010; Zheng et al. 2012).

The function of flavonoids in protecting plants against microbial invasion does not only involve their presence in plants as constitutive agents but also their accumulation as phytoalexins in response to microbial attack. Because of their widespread ability to inhibit spore germination in plant pathogens, they have been proposed also for use against fungal pathogens in humans (Harborne and Williams 2000).

In vitro experimental screening of potential antibacterial and antifungal compounds from plants may be performed with pure substances or crude extracts. The methods used for the two types of organisms are similar. The two most commonly

used screening methods to determine the antimicrobial susceptibility are the broth dilution assay and the disk or agar well diffusion assay. Antifungal phytochemicals can be also analyzed by the spore germination assay (Cowan 1999).

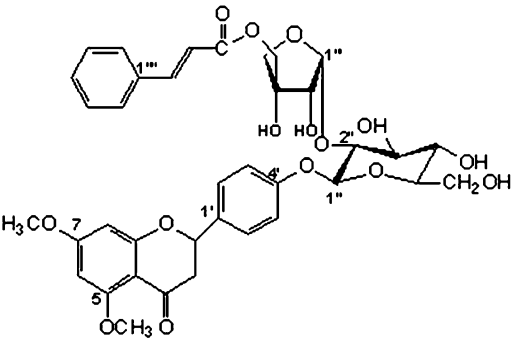
Sharma et al. (2011) studied the antimicrobial activities from the leaf and seed extracts of *Pongomia pinata* and *Vitex negundo*. The antifungal activity was screened against *Candida albicans* and *Trichoderma viride*. The results showed good antifungal activity of all leaf and seed extracts of *Pongomia pinata* and *Vitex negundo* tested.

Many biological activities have been reported in *Slerocarya birrea* (A. Rich) Hochst., subspecies of Anacardiaceae, which is a commonly used plant in Africa. The antifungal properties of this plant containing flavonoids as secondary metabolites was reviewed by Ojewole et al. (2010).

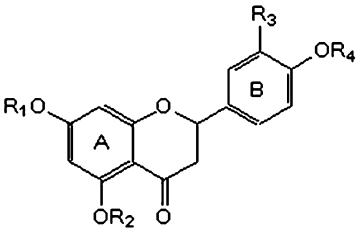
Antibacterial and antifungal activities of plant-derived flavonoids representing two different structural groups were evaluated against *C. albicans* and *Candida krusei* and their drug-resistant isolates using the microdilution broth method. The compounds after extraction were tested and showed high antimicrobial activities employing the microdilution method. The antifungal activities of the compounds tested showed that these compounds presented similar minimal inhibitory concentrations against *C. albicans* compared to the control ketoconazole and fluconazole. The compounds also exhibited a higher degree of antifungal activity against *C. krusei* as compared with fluconazole. The mechanism of action of the compounds should be further investigated. The structures of investigated compounds are shown (Fig. 10.4) (Orhan et al. 2010).

The antifungal effect of total flavonoid extracts from *Patrinia villosa* on *Microsporum gypseum*, *Trichophyton mentagrophytes*, and *Trichophyton rubrum* were studied. The effects of antifungal activity of *P. villosa* on *Trichophyton* was more efficient when compared to *Microsporum*, however less effective than the commercially available control drug ketoconazole (Zheng et al. 2012).

The antimicrobial activity as well as cytotoxicity of 18 prenylated flavonoids (Fig. 10.5) isolated from medicinal plants were evaluated against four bacterial and two fungal (*C. albicans, Saccharomyces cerevisiae*) microorganisms. For the antifungal activity the results of this work were categorized into five subgroups based on the activity spectrum as shown: (1) strong antifungal and antibacterial activity by papyriflavonol A, kuraridin, sophoraflavanone D, and sophoraisoflavanone A, (2) strong antibacterial activity by kuwanon C, mulberrofuran G, albanol B, kenusanone A, and sophoraflavanone G, (3) specific activity against Gram positive bacteria by morusin, sanggenon B and D, kazinol B, kurarinone, kenusanone C, and isosophoranone, (4) moderate activity against *C. albicans* shown by broussochalcone A, and (5) no antimicrobial activity observed for 5-methylsophoraflavanone B. The results suggested that most of the prenylated flavonoids possess a good potential as antimicrobial agents. The authors also suggested that although the pharmacological mechanisms underlying the antimicrobial action of these compounds are unknown, these prenylated flavonoids may act by damaging the membrane and/or cell wall function of the microorganisms (Sohn et al. 2004).



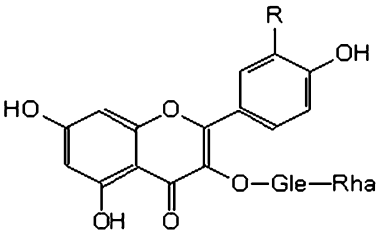
General structure of compounds



R1= R2= CH3; R3 = H; R4 = Glc (5,7-dimethoxyflavanone-4'-0–β-D-glucopyranoside)

R1 = R2 = H; R3 = OH; R4= Glc (5,7,3'-trihydroxy-flavanone-4'0-β-D-glucopyranoside)

R1 = Glc; R2 = R3 = R4 = H (naringenin-7-0–β-D-glucopyranoside)



R = OH (rutin)

R = H (nicotiflorin)

Fig. 10.4 Structural variation of the flavonoid compounds with antifungal activity

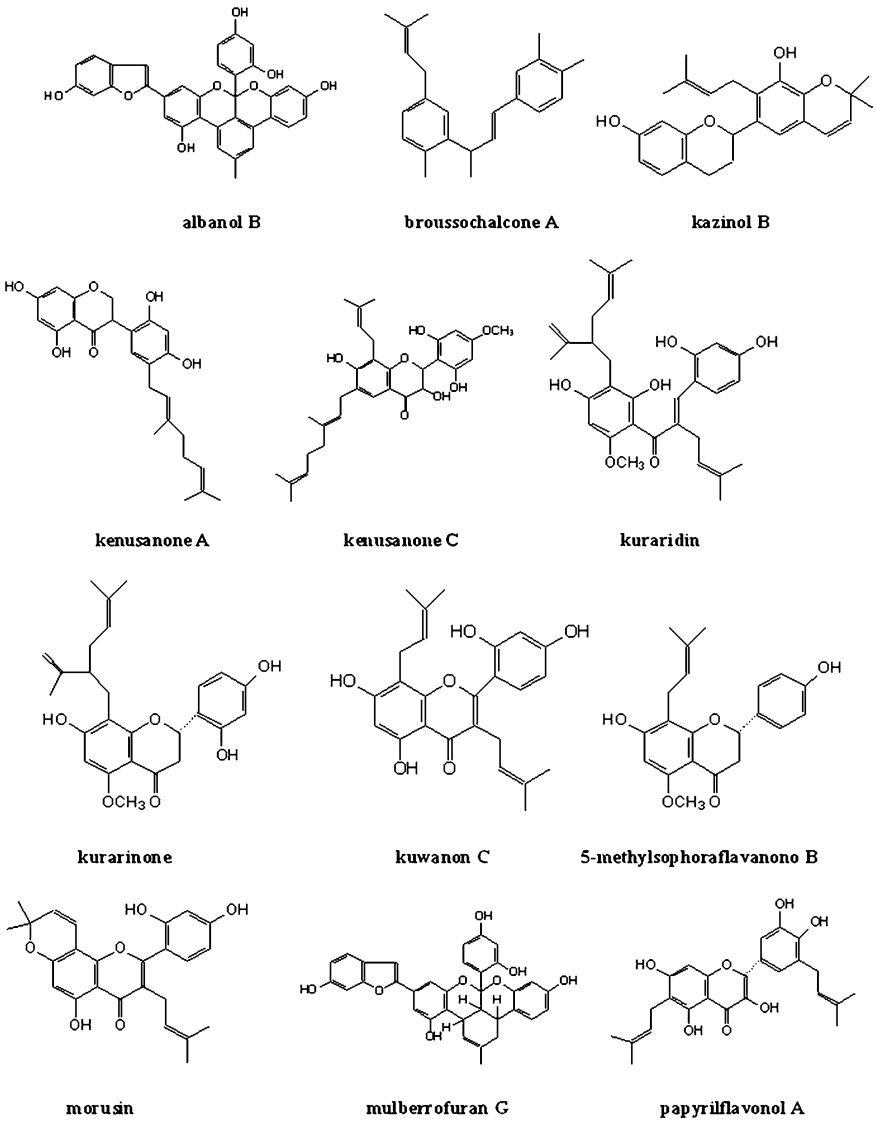
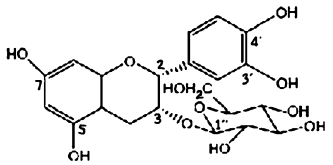


Fig. 10.5 Prenylated flavonoids

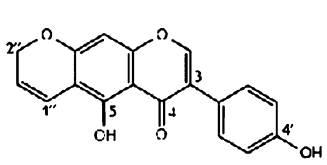
Flavonoids extracted from mango (*Mangifera indica* L.) leaves were tested against five fungal species, *Alternaria alternata* (Fr.) Keissler, *Aspergillus fumigatus* Fresenius, *Aspergillus niger* van Tieghem, *Macrophomia phaseolina* (Tassi) Goid and *Penicillium citrii*. The flavonoids (-)-epicatechin-3-0-glucopyranoside, 5-hydroxy-3-(4-hydroxylphenyl)pyrano[3,2-g]chromene-4(8H)-one, 6-(phydroxybenzyl)taxifolin-7-0-β-D-glucoside, quercetin-3-0-α-glucopyranosil-(1-2)-β-D-glucopyranoside, (-)-epicatechin-2-(3,4-dihydroxyphenyl)-3,4-dihydro-

2H-chromene-3,5,7-triol (Fig. 10.6) showed high antifungal activity against all five fungal species, and at the highest concentration of (1,000 ppm) the five flavonoids reduced the growth of the different target fungal species by 63–97, 56–96, 76–99,76–98, and 82–96 %, respectively (Kanwal et al. 2011).

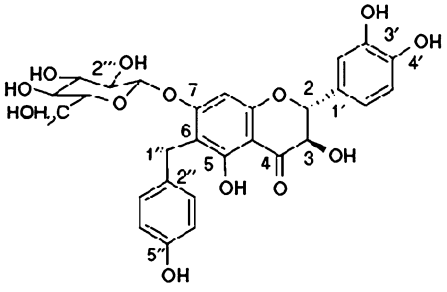
Kanwal et al. (2011) screened the antimicrobial activity of flavonoids isolated from *Azadirachta indica* A. Juss leaves. The antifungal activity of two isolated flavonoids genistein 7-0-glucoside and epicatechin were evaluated against 5 fungal species (*A. alternata, A. fumigatus, A. niger, P. citrii, and M. phaseolina*) and the bacteria species *Lactobacillus* sp., *Escherichia coli*, *Azospirillium lipoferum* and *Bacillus* sp. Analysis of the variance showed that there were highly significant difference in antifungal activity of the two tested flavonoids. Similarly, the effect of concentration was studied as well as response of various species. However in general it was found that in all tested concentrations, both flavonoids significantly reduced the growth of all five test fungal species.



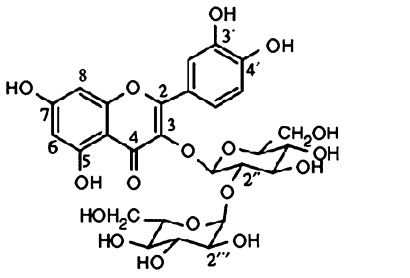
(-)-epicatechin-3-O—glucopyranoside



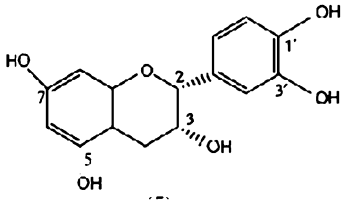
5-hydroxy-3-(4-hydroxylphenyl)pyrano[3,2-g]chromene-4(8H)-one



6-(phydroxybenzyl)taxifolin-7-O-β-D-glucoside (tricuspid)



quercetin-3-O-β-glucopyranosyl-(1-2)-β-D-glucopyranoside



(–)-epicatechin (2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol

Fig. 10.6 Flavonoids extract from mango leaves

A series of 61 Indian medicinal plants were screened for antimicrobial activity in various bacterial strains and three fungal strains (*C. albicans, A. niger,* and *S. cerevisiae*). The results obtained in the study showed that the crude extracts of *Dorema ammoniacum, Sphaeranthus indicus, Dracaena cinnabari, Mallotus philippinensis, Jatropha gossypifolia, Aristolochia indica, Lantana camara*, *Nardostachys jatamansi, Randia dumetorum*, and *Cassia fitula* exhibited a broad spectrum of antimicrobial activity and properties that support the folkloric use of these plants (Kumar et al. 2006).

Wächter et al. (1999) screened plants with biomedical use from south Texas and isolated from the methanolic-dichoromethane extract of *Eysenhardtia texana* Kunth novel flavanones 4',5,7-trihydroxy-8-methyl-6-(3-methyl-[2-butenyl])-(2S)-flavanone, and 4',5,7-trihydroxy-6-methyl-8-(3-methyl-[2-butenyl])-(2S)-flavanone, as well as previously reported 4',5-dihydroxy-7-methoxy-6-(3-methyl-[2-butenyl])-(2S)-flavanone (Fig. 10.7), which showed antifungal properties in *C. albicans*.

The aqueous-ethanolic fraction from extractions of *Cheilanthes dubia* which has been used as a traditional medicine by some ethnic groups of the central Himalaya showed antifungal activity against *A. niger*. From the active fraction of C. *dubia*, three flavonol probably responsible for the activity against *A. niger*, were isolated and identified as quercetin-3,7-dimethyl ether, quercetin-3-metyl ether, and kaempferol-3,4'-dimethyl ether (Fig. 10.8), by (Verma and Kabdwal 2010).

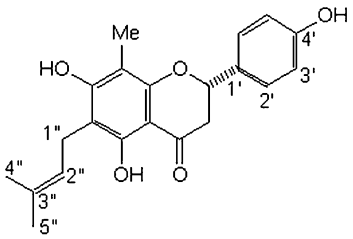
The flavonoids eriodictyol, homoeriodictyol, dihyroquercetin, and luteolin isolated from *Ficus samentosa* var. Henry (King) Comer, were found to be effective against the pathogenic fungi, *Fusarium graminearum*, pumpkin *Fusarium*- Wilt *Curvularia lunata* (Wakker) Boed, *Septoria zeicola* Stout, *Botrytis cinerea*, and *Rhizoctonia solani* (Wang et al. 2010).

Tapas et al. (2008) in a review article on flavonoids such as nutraceuticals stated that a number of flavonoids isolated from the peelings of tangerines tested for the fungistatic activity towards *Deuterophoma tracheiphila* were found to be active; nobiletin and langeretin (Fig. 10.9) exhibited strong and weak activities, respectively, while hesperidin could stimulate fungal growth slightly.

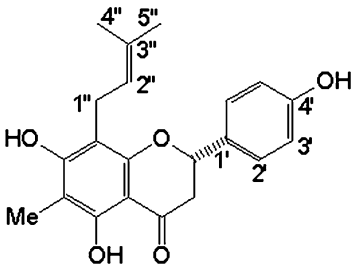
Impatiens balsamina L. Stems, a herbaceous plant of the Balsaminaceae family, has been used traditionally in Chinese medicine. The antioxidant and antimicrobial activities as well as the quantity of phenolic compound substances of this plant, has been studied by Su et al. (2012). Extraction with various solvents showed that all the solvent extracts presented antioxidant activity. The plant extracts were also used to evaluate the antimicrobial activity against the fungal strains, *Penicillium italicum, Penicillium digitatum, A. niger, Aspergillus oryzae, S. cerevisiae,* and *C. albicans*. The *I. balsamina* extracts showed good antifungal efficiency against the selected food-borne pathogens.

Three flavanones 5,7-dihydroxyflavanone (pinocembrin), 7-hydroxy-5-methoxiflavanone (alpinetin) and 5,7-dimethoxyflavanone (chrysin dimethyl ether), and the flavones 5,7-dhydroxiflavone (chrysin) (Fig. 10.10) were isolated and identified from the Combretaceae tree. The compounds studied showed antifungal activity against *C. albicans* and *Proteus vulgaris* as well as antibacterial activity against many bacterial strains (Katerere et al. 2012).

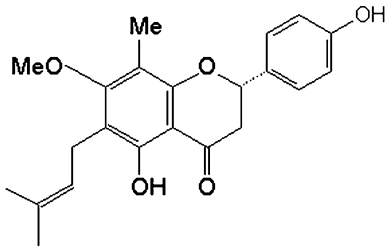
Fig. 10.7 Flavonoids extracted from *Eysenhardtia texana* Kunth



(4′,5,7-trihydroxy-8-methyl-6-(3-methyl-[2-butenyl])-(2S)-flavanone

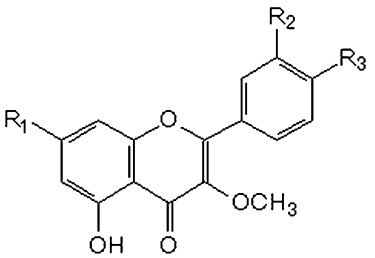


(4′,5,7-trihydroxy-6-methyl-8-(3-methyl-[2-butenyl])-(2S)-flavanone



4′,5-dihydroxy-7-methoxy-6-(3-methyl-[2-butenyl])-(2S)-flavanone

Fig. 10.8 Flavonoids isolated from *C. dubia* (Verma and Kabdwal 2010)



$R_1 = OCH_3$, $R_2 = OH$, $R_3 = OH$ (quercetin-3,7-dimethyl ether)

$R_1 = OH$, $R_2 = OH$, $R_3 = OH$ (quercetin-3-methyl ether)

$R_1 = OH$, $R_2 = H$, $R_3 = OCH_3$ (kaempferol-3,4′-dimethylether)

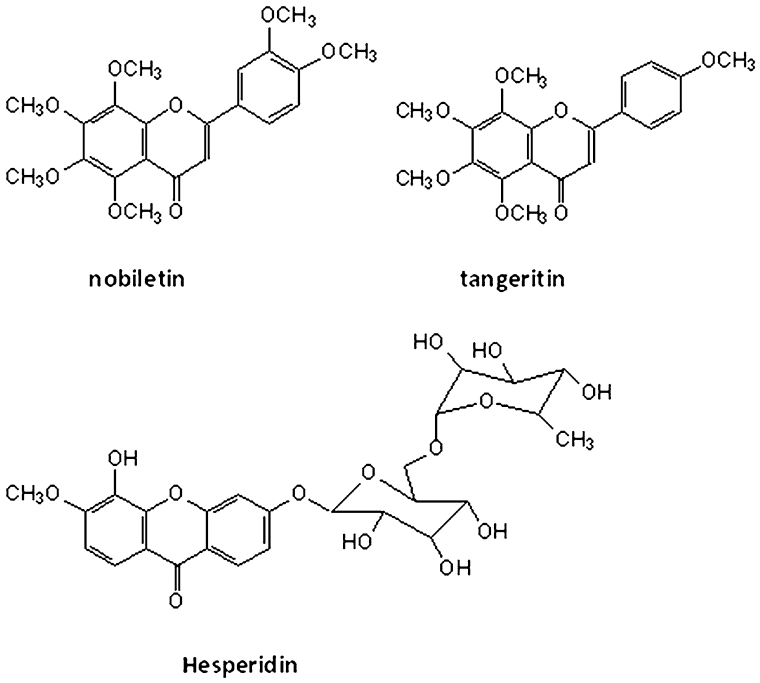


Fig. 10.9 Flavonoids isolated from tangerine orange

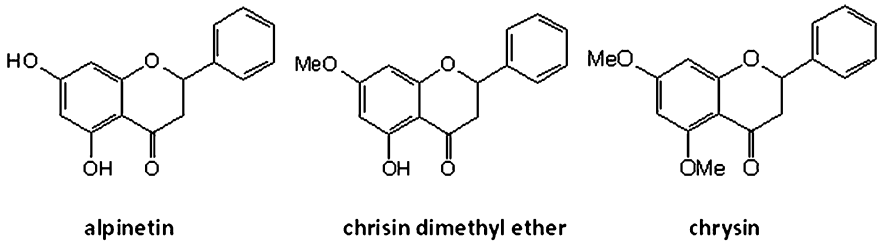


Fig. 10.10 Flavonoids isolated from *Combretaceae* species tree

The antibacterial, inflammatory, antiparasitic, and cytotoxic activities of *Laennecia confusa* were investigated by Martínez Ruiz et al. (2012). The extract fractions of *L. confusa* presented good activity against *C. albicans* and *Cryptococcus neoformans* fungal strains.

Pandley et al. (2010) evaluated the antifungal activity of leaves and bark extracts of *Cinnamomum zeylanicum* against the pathogenic and spoilage fungi, *Aspergillus flavus, A. fumigatus, A. niger, Penicillium* sp., *and C. albicans*. This work suggested that good antifungal, low minimal fungicidal concentration values, and marked antioxidant activity of some extract fractions of *C. zeylanicum* could

be attributed to the presence of differential amount of phenolics, flavonoids, tannins, and terpenoids present in the extracts.

10.5 Concluding Remarks and Perspectives

Flavonoids are present in nature in a wide range of medicinal plants, grains, fruits, vegetables, and flowers. The intake in the daily diet is highly recommended. The large amount of bioactive flavonoid molecules in nature makes them an important and interesting class of compounds for many biological investigations. Nowadays, the flavonoids have extensively been studied mainly because of their broad therapeutical actions which have been proved when experimentally tested in vitro, in vivo, and also in human clinical trials.

The biological activities are due to their structural and chemical similarities with a great range of biomolecules, which gives them a great potential as new molecules of therapeutic interest.

Although the great interest of the scientists in the search for new therapeutic uses of flavonoids as antimicrobial agents is well known, their mechanism of action is still unclear and studies concerning their role in biological interactions, their bioavailability, pharmacokinetics, physiological processes should be further investigated for a better understanding on molecular targets.

The antifungal activity of flavonoid compounds has greatly contributed to the therapeutic benefits of medicinal plants. The flavonoid biosynthetic pathway has been one of the most extensively studied metabolic systems in plants. This will allow the production of structural analogues of active flavonoids through genetic manipulation to be of remarkable value on the search for new therapeutically active molecules.

In addition, the increasing investigations concerning the mechanisms of action of new flavonoid molecules discovered is a promising area of research.

Information about the large variety of flavonoid compounds in nature, their wide range of applications as potent therapeutic agents, the increased research found in the literature on the flavonoids chemistry, and mechanism of action, might provide new commercially available flavonoid molecules which could be used for many therapeutic purposes.

References

Ahmidi F, Sadeghi S, Nodarresi M, Abiri R, Mikaeli A (2010) Chemical composition, in vitro anti-microbial, antifungal and antioxidant activities of essential oil and methanolic extracts of *Hoymenociater longiflones* Benth. Food Chem Toxicol 48(5):1137–1144

Bassetti M, Ginocchio F, Mikulska M (2011) New treatment option against gram-negative organisms. Crit Care 15(215):1–9

Batista LM, Almeida ABA, Magri LP, Toma W, Calvo TR, Vilegas W, Souza –Brito ARM (2004) Gastric antiulcer activity of *Syngonanthus arthrotrichus* silveira. Biol Pharm Bull 27(3):328–332

Bueno JM, Ramos-Escudero F, Sáez-Plaza P, Munhoz AM, Navas MM, Asuero AG (2012) Analysis and antioxidant capacity of anthocyanin pigments. Part I: general considerations concerning polyphenols and flavonoids. Crit Rev Anal Chem 20(2):102–125

Buer CS, Imin N, Djordjevic A (2010) Flavonoids: new roles for old molecules. J Integr Plant Biol 52(1):98–111

Cesco S, Mimmo T, Tonon G, Tomasi N, Pinton R, Terzano R, Neumann G, Weisskopf L, Renella G, Landi L, Nannipieri P (2012) Plant-borne flavonoids released into rhizosphere: impact on soil bio-activities related to plant nutrition. A review. Biol Fertil Soils 48(2):123–149

Cowan MM (1999) Plant products as antimicrobial agents. Clin Microbiol Rev 12(4):564–582

Cushinie TTP, Lamb AJ (2005) Antimicrobial activity of flavonoids. Int J Antimicrob Agents 26(5):343–356

Estelle G, Edwige N, Ahcere B (2011) Flavonoids as antimicrobial agents: recent progress and state of the art. Curr Org Chem 15(15):2608–2615

Fraga CG, Galleano M, Verstraeten SV, Oteiza PI (2010) Basic biochemical mechanisms behind the health benefits of polyphenols. Mol Aspects Med 31(6):435–445

Friedman M (2007) Overview of antimicrobial, antitoxin, antiviral, and antifungal activities of tea flavonoids and teas. Mol Nutr Food Res 51(1):116–134

Harborne JB, Williams CA (2000) Advances in flavonoid research since 1992. Phytochemistry 55(6):481–504

Himesh S, Sarvesh S, Sharan PS, Mishra K, Singai AK (2011) Preliminary phytochemical screening and HPLC analysis of flavonoid from methanolic extract of leaves of *Annona squamosal*. Int Res J Pharm 2(5):242–246

Jagtap UB, Bapat VA (2010) Artocarpus: a review of its traditional uses, phytochemistry and pharmacology. J Ethnopharmacol 129(2):142–166

Kaefer CM, Milner JA (2008) The role of herbs and species in cancer prevention. J Nutr Biochem 19(6):347–361

Kanwal Q, Hussain I, Siddiqui HL, Javaid A (2011) Antimicrobial activity screening of isolated flavonoids from Azadirachta indica leaves. J Serb Chem Soc 76(3):375–384

Karimi E, Jafar HZE, Ahmad S (2011) Phenolics and flavonoids profiling and antioxidant activity of three varieties of Malaysian indigenous medicinal herb *Labisia pumila Benth*. J Med Plants Res 5(7):1200–1206

Katerere DR, Gray AI, Nash RJ, Waigh RD (2012) Phytochemical and antimicrobial investigation of stilbenoids and flavonoids isolated from three species of Combretaceae. Fitoterapia 83(5):932–940

Kyselova Z (2011) Toxicological aspects of the use of phenolic compounds in disease prevention. Interdiscip Toxicol 4(4):173–183

Krishna PM, Rao KNV, Sandhya S, Banji D (2012) A review on phytochemical, ethnomedicinal and pharmacological studies on genus *Sophora* Fabaceae. Braz J Pharmacog 22(5):1145–1154

Kuete V, Wiench B, Hegazy MEF, Mohamed TA, Fankan AG, Shahat AA, Efferth T (2012) Antibacterial activity and cytotoxicity of selected egyptian medicinal plants. Planta Med 78(2):193–199

Kumar PV, Chauhan NS, Padh H, Rajani M (2006) Search for antibacterial and antifungal agents selected Indian medicinal plants. J Ethnopharmacol 107:182–188

Lakshmipathy DP, Kannabiran K (2010) Review on dermatomycosis: pathogenesis and treatment. Nat Sci 2(7):726–731

Martínez Ruiz MG, Richard-Greenblatt M, Juárez ZN, Av-Gay Y, Bach H, Hernández LR (2012) Antimicrobial, anti-inflammatory and cytotoxic activities of *Laennecia confuse*. Sci World J 2012:1–8

Ndhlala AR, Moyo M, Van Staden J (2010) Natural antioxidants: fascinating or mythical biomolecules? Molecules 15(10):6905–6930

Nijveldt RJ, van Nood E, van Hoorn DEC, Boelens PG, van Norren K, van Leeuwen PA (2010) Flavonoids: a review of probable mechanisms of action and potential applications. Am J Clin Nutr 74(4):418–425

Ojewole JAO, Mawoza T, Chiwororo WDH, Owira PMO (2010) *Sclerocarya birrea* (A. Rich) Hochst. ['Marula'] (Anacardiaceae): a review of its phytochemistry, pharmacology, and toxicology and its ethnomedicinal uses. Phytother Res 24:633–639

Orhan DD, Özcelik B, Özgen S, Ergum F (2010) Antibacterial, antifungal and antiviral activities of some flavonoids. Microbiol Res 165(6):496–504

Pandley AK, Mishra AK, Mishra A, Kumar S, Chandra A (2010) Therapeutic potential of *C. zeylanicum* extracts: an antifungal and antioxidant perspective. Int J Biol Med Res 1(4):228–233

Patel JR, Tripathi P, Sharma V, Chauhan NS, Dixit VK (2011) *Phyllanthus amarus*: ethnomedicinal uses, phytochemistry and pharmacology: a review. J Ethnopharmacol 138(4):286–313

Pierson JT, Dietzgen RG, Shaw PN, Roberts-Thomson S, Montheith GR, Gidley J (2012) Major Australian tropical fruits biodiversity: Bioactive compounds and their bioactivities. Mol Nutr Food Res 56(3):357–387

Pinheiro PF, Justino GC (2010) Structural analysis of flavonoids and related compounds—a review of spectroscopic applications. In: Rao V (ed) Phytochemicals—A global perspective of their roles in nutrition health, InTech. CRC press, Boca Raton, USA

Ponnusamy K, Petchiammal C, Mohankumer R, Hopper W (2010) In vitro antifungal activity of indirubin isolated from South Indian ethnomedicinal plant Wrightia tinctoria R. Br. J Ethnopharmacol 132(1):349–354

Recio MC, Rios JL (1989) A Review of Some Antimicrobial Compounds Isolated from Medicinal Plants Reported in the Literature 1978–1988. Phythother Res 3(4):125

Rios JL, Recio MC (2005) Medicinal plants and antimicrobial activity. J Ethnopharmacol 100:80–84

Rokaya MB, Münzbergová Z, Timsina B, Bhattarai KR (2012) *Rheum austral* D. Don: a review of its botany, ethnobotany, phytochemistry and pharmacology. J Ethnopharmacol 141(3):761–774

Ruddock PS, Charland M, Ramirez S, López A, Towers N, Arnason JT, Liao M, Dillon JAR (2011) Antimicrobial activity of flavonoids from *Piper Ianceaefolium* and other Colombian medicinal plants against antibiotic susceptive and resistant strains of *Neisseria gonohhoeae*. Sex Transm Dis 38(2):82–88

Sandhar HK, Kumar B, Prasher S, Tiwari P, Salhan M, Sharma P (2011) A review of phytochemistry and pharmacology of flavonoids. Int Pharm Sci 1(1):25–37

Sharma A, Tyagi S, Nag R, Chaturvedi A, Nag TN (2011) Antimicrobial activity and celular toxicity of flavonoid extracts from *Pongamia pinnata* and *Vitex negundo*. Rom Biotechnol Lett 16(4):6396–6400

Sher A (2009) Antimicrobial activity of natural products from medicinal plants. Gomal J Med Sci 7(1):72–78

Sohn HY, Son KH, Kwon CS, Kwon GS, Kang SS (2004) Antimicrobial and cytotoxic activity of 18 prenylated flavonoids isolated from medicinal plants: *Morus alba* L., *Morus mongolica* Schneider, *Broussnetia papyrifera* (L.) Vent, *Sophora flavescens* Ait and *Echinosophora koreensis* Nakai. Phytomedicine 11(7/8):666–672

Su BL, Chen JY, Chen CY, Guo JH, Chang-Gan H (2012) Antioxidant and antimicrobial properties of various solvent extracts from *Impatiens balsamina* L. stems. J Food Sci 77(6):C614–C619

Tapas AR, Sakarkar DM, Kakde RB (2008) Flavonoids as nutraceuticals: a review. Trop J Pharm Res 7(3):1089–1099

Verma DL, Kabdwal L (2010) Flavonoids from *Cheilanthes dubia Hope*. Nat Sci 8(10):306–310

Wächter GA, Hoffmann JJ, Furbacher T, Blake ME, Timmermann BA (1999) Antibacterial and antifungal flavonones from *Eysenhardtia texana*. Phytochemistry 52(8):1469–1471

Wang X, Wei X, Tian Y, Shen L, Shu H (2010) Antifungal flavonoids from *Ficus sarmentosa* var. *henry* (King) corner. Agr Sci China 8(5):690–694

Wink M, Ashour ML, El-Readi (2012) Secondary metabolites from plants inhibiting ABC transporters and microbes to cytotoxic and antimicrobial agents. Front Microbiol 3(130):1–15

Yang N, Kim DJ, Kim BH, Sohn M, Choi MJ, Choi YH (2010) Aloe-iduced toxic hepatitis. J Korean Med Sci 25:492–495

Zampini IC, Villena J, Salva S, Herrera M, Isla MI, Alvarez S (2012) Potentiality of standardized extract and isolated flavonoids from *Zuccagnia punctata* for the treatment of respiratory infections by Streptococcus pneumonia: *in vitro* and *in vivo* studies. J Ethnopharmacol 140(2):287–292

Zheng N, Wang Z, Shi Y, Lin J (2012) Evaluation of the antifungal activity of total flavonoids extract from *Patrinia villosa* Juss and optimization by response surface methodology. Afr J Microbiol Res 6(3):586–593

Part III
Plants used in Ayurveda and Traditional Systems for Fungal Diseases

Chapter 11
Antifungal Metabolites from Medicinal Plants used in Ayurvedic System of Medicine in India

Ajay Kumar Meena, Shahin Khan, Mruthyumjaya Meda Rao, Radha Krishna Reddy and Madhan Mohan Padhi

Abstract India has rich heritage of using medicinal plants in traditional medicine such as Ayurveda, Siddha, and Unani besides folklore practices. Ayurvedic system of medicine has its long history of therapeutic potential. The use of both plant extracts and phytochemicals with known antifungal properties is of great significance. The increasing failure of chemotherapeutics and antifungal resistance exhibited by pathogenic microbial agents has led to the screening of several medicinal plants for their potential antimicrobial activity. The most important bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds. Phytomedicines derived from plants have shown great promise in the treatment of various fungal diseases. Single and poly herbal preparations have been used for the treatment of various types of illnesses. Interest in plants with antifungal properties has revived as a result of current problems associated with the use of chemically synthesized antifungals. The aim of present communication is to summarize the antifungal agent and metabolite from plants. Human fungal infections are increasing due to the increased cancer and AIDS patient. Infection with HIV leads to immune suppression and up to 90 % of HIV infected individuals contract fungal infections of which 10–20 % die as a direct consequence of these infections.

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11.1 Introduction

Fungal diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide (Wealth of India 1998). Human infections, particularly those involving the skin and mucosal surfaces, constitute a serious problem, especially in tropical and subtropical developing countries (Portillo et al. 2001). In humans, fungal infections range from superficial to deeply invasive or disseminated, and have increased dramatically in recent years. The treatment of mycoses has lagged behind bacterial chemotherapy and fewer antifungal than antibacterial substances are available. Therefore, a search for new antifungal drugs is extremely necessary (Fortes et al. 2008). During the past several years, there has been an increasing incidence of fungal infections due to a growth in immune compromised population such as organ transplant recipients, cancer, and HIV/AIDS patients. This fact coupled with the resistance to antibiotics and with the toxicity during prolonged treatment with several antifungal drugs (Giordani et al. 2001) has been the reason for an extended search for newer drugs to treat opportunistic fungal infections (Fostel et al. 2000). In developing countries, microorganisms are frequently a cause of prevailing diseases, presenting a serious public health issue in a significant segment of the population as uncovered by either private or official health care systems. An antifungal drug is a medication used to treat fungal infections such as athlete's foot, ringworm, candidiasis (thrush), serious systemic infections such as cryptococcal meningitis, etc. Such drugs are usually obtained by a doctor's prescription or purchased over-the-counter. But use of this type of drugs used in large way makes the unusable due to resistance to antibiotics and with the toxicity during prolonged treatment. There are large numbers of drawback in synthetic drugs so people move toward herbal drugs which are safer. Yeasts of the genus *Candida* (in particular *C. albicans*) and of the species *Cryptococcus neoformans* are the fungal agents most frequently involved in the etiology of infectious processes in subjects affected by AIDS. Many studies investigating the antifungal susceptibility of clinical strains of *Candida* spp. have been performed with a variety of results and these studies point to the emergence of new resistant strains (Resende 1999). Disseminated cryptococcosis, on the other hand, affects a more limited percentage of patients (6–8 %), yet is still a serious life-threatening condition. In the present scenario, an emergence of multiple drug resistance in human pathogenic fungi and the small number of antifungal classes available stimulated research toward the discovery of novel antifungal agents from other sources, such as medicinal plants (Chen et al. 1996; Fostel and Lartey 2000; Elisabetsky and Souza 2002; Fortes et al.2008).

11.2 Important Antifugal Plants and Ayurvedic Drugs

Ayurvedic system of medicine has its long history of therapeutic potential. The use of both plant extracts and phytochemicals with known antimicrobial properties is of great significance. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Rojas et al. 2003). Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds that are a part of the essential oils (Jansen et al. 1987) as well as tannin (Saxena et al. 1994). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds. Traditional medicine relies on many plants, and many current medicines have been developed from plants. Phytomedicines derived from plants have shown great promise in the treatment of various diseases including viral infections. Single and poly herbal preparations have been used throughout history for the treatment of various types of illness. Interest in plants with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics (Kavya et al. 2007). In India, a number of plant extracts are used against diseases in various systems of medicine such as Ayurveda, Unani, and Siddha. Only a few of them have been scientifically explored. Plant-derived natural products have received considerable attention in recent years due to their diverse pharmacological activities. The ayurvedic approach to the prevention and treatment of microbial infection recognizes the emergency use of modern drugs, but recommends traditional herbal combinations and extracts known to balance the individual and improve health, as well as herbs that help to combat or prevent microbial infections. The Indian plants possessing significant antimicrobial activity are neem, long pepper fruit, *Tinospora cordifolia* (Willd.) Hook. F. and Thomson, and *Emblica officinalis* Gaertn. among others (Treadway 1998). The antibacterial and antifungal activity of powders of single herbs (Amalaki and Yastimadhu churna) and two ayurvedic formulations containing combination of herbs (DN-90 and Asanadi Kwatha Churna) against bacteria and fungi has been studied. Result of antibacterial activity of Amalaki churna was comparable with standard drug Streptomycin. Asanadi Kwatha Churna inhibited bacteria to more extent than Yastimadhu churna and DN-90. Among fungi tested, more antifungal activity was observed against Mucor Sp. (Prashith et al. 2010). Medicinal plants represent a rich source of antimicrobial agents (Mahesh and Satish 2008). Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine (Mann et al. 2008). Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides, and many pharmaceutical drugs. Plant products still remain the principal source of pharmaceutical agents used in traditional medicine

(Ibrahim 1997; Ogundipe et al. 1998). The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world (Reddy et al. 2001; Ateb and ErdoUrul 2003).

Ethyl acetate extract of *Taenia asiatica* showed significant antifungal activity against most of the tested fungi. The lowest MIC was observed against *Trichophyton rubrum* (0.62 mg/mL). n-Hexane and methanol extracts did not inhibit many tested fungi. Antibacterial and antifungal activity of essential oil from this plant has been reported (Saxena and Sharma 1999; Jeevan et al. 2004; Liu et al. 2009). The strongest effect was reported for *Eupatorium rotundifolium* and *Terminalia triora*, *T. mentagrophytes* and *T. rubrum* being the most susceptible species with MICs ranging from 100 to 250 μg/mL. The maximum antifungal activity is in ethyl acetate extract of *C. fistula* (flower), *T. asiatica* (leaves), and *n*-Hexane extract of *A. tetracantha* (leaves) (Duraipandiyan and Ignacimuthu 2011). Water extracts of *Acacia nilotica*, *Justicia zelanica*, *Lantana camara,* and *Saraca asoca* exhibited good activity against all the bacteria tested and the MIC was recorded in range of 9.38–37.5 and 75–300 μg/ml against the bacterial and fungal pathogens, respectively (Dabur et al. 2007). Four plants including *Grewia arborea*, *Melia azedarach*, *Peltophorum pterophorus,* and *Terminalia chebula* showed exceptionally prominent activity. Methanolic extract of plant *Grewia arborea* showed maximum activity even at lower concentrations (100 mg/ml) (Bobbarala et al. 2009).

Curcuma longa Linn. leaf oil showed antifungal activity against six phytopathogenic fungi even in 2 μl/disk concentrations. All six fungi were found sensitive to this oil, *Macrophomina phaseolina* and *Botrydiplodia theobromae* showed more inhibition (Chowdhry et al. 2005). Ayurvedic medicines Haridra khand, Mahasudarshan kwath, Prasadan, Sanhanan, Seera have *C. longa* as a main ingredient. These formulations are used in bacterial and fungal infections. Various extracts of *Swertia chirata* known as Chirayta have been studied for antibacterial and antifungal activity. Tests showed inhibitory activity against various gram positive and gramnegative bacteria. The study also showed moderate inhibitory action on various fungi (Awasti et al. 2005). *Swertia chirata* is main ingredient of Bhunumbadi kwath, Bhunimadi vati ayurvedic medicines. Chemical analysis and antimicrobial activity of the essential oil of *Eucalyptus cinerea* (Myrataceae) has been investigated. The essential oil showed antimicrobial activity against yeast and *Candida albicans*. Nilgiri oil is used as ayurvedic formulation in fungal infection (Franco et al. 2005). The aqueous extract of *Peganum harmala* seeds (wildrue) exhibited significant antibacterial and antifungal activity against most of the phytopathogenic fungi tested (Kumar et al. 2004). Antifungal activity of *P. harmala* on 6 and 3 species of Candida and *Aspergillus,* respectively, was evaluated in vitro. Alcoholic extract of *P. harmala* seeds showed; MIC of 0.312 mg/ml on *Candida glabrata* and MIC of 1.25 mg/ml on *C. albicans* as the highest and lowest inhibitory effects, respectively. Moreover, minimum fungicidal concentration of the extract on *Candida* isolates was determined in a range of 0.625–2.5 mg/ml (Diba et al. 2011). Fresh extract of *Tephrosia purpurea* (Sharapunkha) roots was treated for antibacterial and antifungal activity, and thus showed antibacterial

activity. Sharapunkha churna (fine powder) an ayurvedic medicine was used as antifungal ayurvedic medicines (Deshpande et al. 2005). Bioassay-guided fractionation of *Tribulus terrestris* (Gokhru) yielded eight steroid saponins. They showed significant in vitro and in vivo antifungal activity, weakening the virulence of *C. albicans* and killing fungi through destroying the cell membrane (Zhang et al. 2006). Gokshuradiguggulu, Gukshurasav, Gokshuradi ghruta was prepared by using *T. terrestris* (Gokhru) as main ingredient. Ethanolic extract of *Punica granatum* (Dadima-pomegranate) showed activity against three *Candida* species whereas it was ineffective against *C. albicans*. The methanol, acetone, and propanol extracts were effective against *C. albicans*, *Candida krusei*, *Candida parapsilosis*, and *Candida tropicalis* (Nair and Chanda 2005). *Punica granatum* is used to prepare important antifungal ayurvedic formulations like Dadimavelha, Dadimadichoorna, Dadim Twak kwath, Dadimadi ghruta. The ethanolic extracts of *C. longa* (*Turmeric*) (Haridra) and *Alpina galanga* (Rasna) exhibited excellent phytotoxic activity against *Lemna minor* (duckweed). These extracts were also found to possess good antifungal activities against *Trichophyton longifusus* (65 and 60 %, respectively) (Khattak et al. 2005). *Curcuma longa* is used as main ingredient in formulation of Haridra churna, Maharasnadi kwath. Leptospirosis is one of the most widespread zoonosis in the world. It is a recurring epidemic in tropics, especially among those who work in waterlogged areas. Neem oil is traditionally known for its antibacterial and antidermatophytic activities. This was evaluated as a preventative against Leptospiral (Leptospiricidal activity) through skin. Neem oil was found as an effective antibacterial film on skin and prevents the portal entry at bacteria. Oil water solution produced acidic pH (6) and it is leptospiricidal activity. The acidic effect was found up to a radius of 20 cm and persistent throughout while the person in water (Kurian and Thomas 2004). Neem oil is used to prepare Paribhadra oil and Panchnimba churna an Ayurvedic formulations. Ethanolic extracts of *Trigonella foenum-graecum* (leaves) (Methika-fenugreek), *C. longa* (rhizome) (Haridra-Turmuric), *Aloe vera* (aerial part) (Kumari), *Allium sativum* (stem) (Rasona-garlic), *Zingiber officinale* (rhizome) (Shunthi—ginger), and *Centella asiatica* (leaves) (Mandukparni-indian pennywort), exhibited good fungicidal activity (Perumal et al. 2004). Bioactivity at flavonoids *Ocimum sanctum* (Tulsi) were studied and observed that it is useful against *Pseudomonas*, *Staphylococcus* (gram positive), *Escherichia coli*) (gram negative), bacteria, and *C. albicans* (fungus). Tulasi churna (powder), Tulasi kwath are important Ayurvedic formulations which used *O. sanctum* (Tulsi) as main ingredient (Kulkarni 2012).

11.3 Assay of Antifungal Activity

Antifungal assays are regularly used to determine whether plant extracts will have potential to treat human fungal infections (e.g., tinea) or have use in agricultural/horticultural applications. In general these assays are quick, low cost, and easily

access. Activity against filamentous fungi can be evaluated in well diffusion, agar dilution, and broth/micro-broth dilution methods with many of the same limitations and advantages as discussed in Table 11.1. MIC is defined as the lowest concentration of test substance that prevents visible fungal growth. Anti-sporulation activity can be assessed by using scanning electron microscopy, while effects on conidia germination can be evaluated by exposing the conidia to the test substance and subsequently counting the number of conidia with germ tubes equal to 1–1.5 time's conidia length. Additional observations of germinated conidia over a set period will also allow evaluation of the effect of the plant extract on germ tube growth. Inouye and co-workers (2001a, b, c) have investigated the susceptibility of fungi to several essential oils and have shown that MIC values can be calculated using a range of methods. Most significantly, they have shown that when assays are done under closed conditions (i.e., the Petri dish is sealed) the MICs are significantly lower than when performed under open conditions. The action of essential oil and plant extract volatiles on fungal growth has been demonstrated against a range of fungi and has important implications for the screening of plant extracts for antifungal activity. Results in these assays will depend not only on the antifungal activity mediated by direct contact with the test substance but also on the volume of the experimental chamber and whether it is open or closed (and hence the presence and concentration of extract or oil volatiles). The method for evaluating the antifungal activity of extract volatiles is straightforward and involves the placement of a paper disk with test substance on the inverted lid of a Petri dish and subsequent evaluation of fungal growth; however, this is rarely considered in antifungal screening assays. Given the impact that volatiles can have on fungal growth it is recommended that this be included as a standard part of antifungal assessment of plant extracts (Antonov et al. 1997; Inourye et al. 2001a, b, c; Edris and Farrag 2003; Jenny 2006).

11.3.1 Preparation of the Extract for Assay

For alcoholic extractions, plant parts are dried, ground to a fine texture, and then soaked in methanol or ethanol for extended periods. The slurry is then filtered and washed, after which it may be dried under reduced pressure and redissolved in the alcohol to a determined concentration. When water is used for extractions, plants are generally soaked in distilled water, blotted dry, made into slurry through blending, and then strained or filtered. The filtrate can be centrifuged multiple times for clarification (Kubo et al. 1993). Crude products can then be directly used in the drop test and broth dilution micro well assays to test for antifungal and antibacterial properties and in a variety of assays to screen bioactivity. Tween 80 resulted in weaker bioactivity in agar dilution assays. Antifungal assay was altered by modifying the pH of the fungal growth media. As the media pH became more alkaline the *Eucalyptus* essential oil had greater inhibitory effect on the fungi.

Table 11.1 Various methods for antifungal assay with their strengths and limitations

Method for assays	Strengths	Limitations
Disk well diffusion	Low cost	Differential diffusion of extract components due to partitioning in the aqueous media
	Results available in 1–2 days	Inoculum size, presence of solubilizing agents, and incubation temperature can affect zone of inhibition
	Does not require specializes laboratory facilities	Volatile compounds can affect bacterial and fungal growth in closed environments
	Uses equipment and reagents readily available in a microbiology laboratory	Data is only collected at one or two time points
	Can be performed by most laboratory staff	
	Large numbers of samples can be screened	
	Results are quantifiable and can be compared statistically	
Agar diffusion	Low cost	Hydrophobic extracts may separate out from the agar
	Does not require specializes laboratory facilities	Inoculum size, presence of solubilizing agents and incubation temperature can affect zone of inhibition
	Uses equipment and reagents readily available in a microbiology laboratory	Volatile compounds can affect bacterial and fungal growth in closed environments
	Can be performed by most laboratory staff	Data is only collected at one or two time points
		Use of scoring system is open to subjectivity of the observer. Some fungi are very slow growing
Broth diffusion	Allows monitoring of activity over the duration	Essential oils may not remain in solution for the duration of the assay; emulsifiers and solvents may interfere with the accuracy of results
	More accurate representation of antibacterial activity	
	Micro-broth methods can be used to screen large numbers of sample in a cost effective manner	Labor and Time-intensive if serial dilutions are used to determine cell counts
		Highly colored extracts can interfere with colorimetric endpoints in micro broth methods
TLC Bio-autography	Simultaneous determination and fractionation of bioactivity	Unsuitable where activity is due to component synergy
		Dependent on extraction method and TLC solvent used
		Technique is more difficult with fungi because they grow slower and contamination can be a problem

11.3.2 Antifungal Activity Test

Agar diffusion and micro-dilution methods were used to determine the antifungal activities of the medicinal plant extracts against *C. albicans*, *C. krusei,* and *C. neoformans*. Sabouraud dextrose broth (SDB) was used for the preparation of fungal cultures and for the determination of the MIC and was prepared following the manufacturer's instructions. Sabouraud dextrose agar (SDA) was used to determine the activity of the plant extracts against the fungal organisms and was prepared following the instruction of the manufacturer.

11.3.2.1 Agar Diffusion Assay

Using the micropipette, 100 µl of 0.5 Mcfarland solution of *C. albicans* culture (in SDB) was spread over the surface of an agar plate using a sterile hockey stick. The same procedure was followed for *C. krusei* and *C. neoformans*. Using a sterile 5 cm plastic pipette, four holes were punched (2 mm in diameter) in each of the culture plates. In the first hole, 10 µl of the positive control drug was added; 10 µl of DMSO was added as a negative control in the second hole; 5 and 10 µl of the plant extract were added in the third and last holes. The culture plates were then incubated at 37 °C and the results were observed after 1 to 6days depending on the fungi. The clear zone around the plant extract was measured in mm and indicated the activity of the plant extract against the fungal organisms. The experiments were done in triplicate.

11.3.2.2 Microdilution Assay

The micro dilution method was employed to determine the minimum inhibitory concentration (MIC) of the plant extracts using 96 well micro titration plates as previously described (Samie et al. 2005). Briefly, 185 µl of the broth was added into each well in the first row of micro titration plate and 100 µl to the rest of the wells from the second row downwards. 15 µl of the plant extracts was then added into each well on the first row (row A), starting with the positive control (Nystatin, Roche), followed by the negative control (the 20 % DMSO used to dissolve the plant extracts) and the plant extracts in the rest of the wells on that row. A twofold serial dilution was done by mixing the contents in each well of the first row and transferring 100 µl to the second well of the same column and the same was done up to the last well of the same column and the last 100 µl from the last well was discarded. Then 100 µl of yeast suspensions was added. The results were observed after 24 h incubation at 37 °C followed by the addition of 40 µl of a 0.2 % Iodo Nitro Tetrazolium (INT) solution after a further incubation of 4 h at 37 °C. The wells that did not show any color change after INT was added indicating the

concentration of the plant extract that was able to inhibit fungal growth whereas the pink color change indicated fungal growth.

11.3.2.3 Determination of the MFC

The MFC was determined by inoculating the contents from the MIC plates onto SDA plates and the results were observed after 24 h incubation at 37 °C. The presence of the fungal colonies on agar plates was an indication that the plant extract only inhibited fungal growth without killing them and the absence of fungal growth indicated that the plant extract was able to kill the fungal organisms. The lowest concentration of the plant extract that was able to kill the microorganisms was considered as the minimum fungicidal concentration.

11.3.2.4 Time-Kill Experiment

Determination of the rate of kill of the crude extract was done following the procedure described by Aiyegoro et al. (2008) with slight modifications. Briefly, inocula were prepared as described above. The resultant suspension was diluted 1:100 with fresh sterile broth and used to inoculate 50 ml volumes of SDB incorporated with extract at MFC to a final cell density of approximately 2×10^6 cfu/ml. The flasks were incubated at 37 °C on an orbital shaker at 120 rpm. A 500 µl sample was removed from cultures at 0, 2, 5, 10, and 24 h, diluted serially and 100 µl of the diluted samples were placed on SDA plates and incubated at 37 °C for 24 h. Controls included extract free Mueller–Hinton broth seeded with the test inocula (Samie et al. 2010).

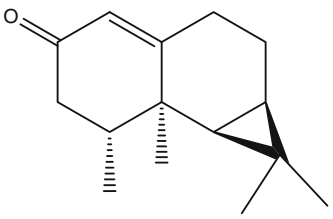
11.3.2.5 TLC Direct Bioautography

In this method TLC is performed using crude extracts, extract fractions, or whole essential oils (Rahalison et al. 1991). The developed TLC plate is then sprayed with, or dipped into, a bacterial or fungal suspension (direct bioautography) or overlain with agar and the agar seeded with the microorganism (overlay bioautography) (Hoult and Paya 1996; Stern et al. 1996; Yemele et al. 2006). This technique may be utilized with either spore-forming fungi or bacteria, and can be used to track activity through a separation process. It is a sensitive assay and gives accurate localization of active compounds. For the assessment of antifungal activity, the plant pathogen *Cladosporium cucumerinum* can be used as it is nonpathogenic to humans, readily forms spores, and can be easily grown on TLC plates with the correct medium. A simple method is outlined as follows:

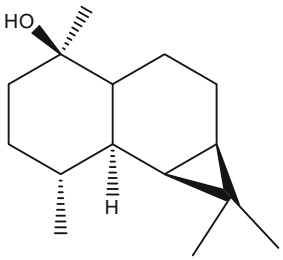
(a) Extracts or pure compounds may be spotted onto analytical TLC plates in duplicate (plastic-backed, Kieselgel 60 PF_{254}, Merck Art 5735), developed with the appropriate mobile phase and dried.
(b) A slope of *C. cucumerinum* is prepared from a culture and allowed to sporulate for two days.
(c) A TLC growth medium is prepared as follows: NaCl (1 g), KH_2PO_4 (7 g), Na_2HPO_4–$2H_2O$ (3 g), KNO_3 (4 g), $MgSO_4$ (1 g), and Tween 80 (20 drops added to water (100 mL). A volume of 60 mL of this solution should be added to 10 mL of aqueous glucose (30 % w/v).
(d) A fungal suspension is prepared by adding the above solution to the fungal slope and shaking it.
(e) The suspension is sprayed onto one of the TLC plates and incubated at 25 °C for 2 days in an assay tray with wet cotton wool to ensure a moist atmosphere.
(f) The inoculated TLC plate is observed at regular intervals, and the presence of antifungal compounds is indicated by inhibition or reduced lack of mycelial growth. This is frequently observed as light spots against a dark green background. The spraying is performed in a lamina flow cabinet.
(g) The remaining TLC plate is visualized using a spray reagent and/or UV detection and compared with the incubated plate.

Aspergillus niger, a more easily sporulating fungus, may be used in the place of *Cladosporium* sp., but care must be taken with this organism because of the risk of aspergillosis, and all microbes should be handled aseptically in a lamina flow cabinet. Controls of antifungal compounds such as amphotericin B should be used each time this assay is performed. This assay does not distinguish between fungicidal and fungi static metabolites and further assays such as a liquid broth assay will need to be performed to measure minimum inhibitory concentration (MIC). It should be noted that amphotericin B is highly toxic and care must be exercised in its use (Cole 1994).

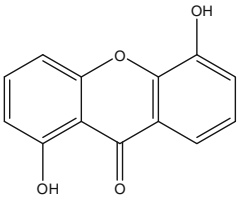
Dellar et al. (1994) isolated the antifungal sesquiterpenes aristolen-2-one **(1)** and prostantherol **(2)** from two species of *Prostanthera* (Labiatae). Activity was assessed and tracked through the separation procedure by the use of direct bioautography with *C. cucumerinum* as the target fungus. Compound inhibited the growth of *C. cucumerinum* for 70 h at a dose of 1 mg, whereas caused inhibition at 10 mg for the same duration. The antifungal activity of many plant phenolic compounds can be readily assessed using this simple procedure. Hostettmann and Marston (1994) have investigated a series of xanthones **(3–5)** from *Hypericum brasiliense* (Guttiferae) for activity against *C. cucumerinum* (Natarajan et al. 2001). One of these compounds (xanthone 1) exhibited a low inhibitory dose (25 mg), which may warrant further investigation of the antifungal function of these interesting compounds.



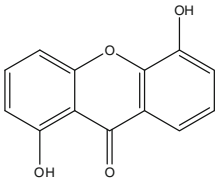
Aristolene-2-one (1)



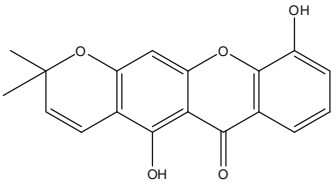
Prostantherol (2)



Xanthone 1(3)



Xanthone 2 (4)



Xanthone 3 (5)

11.3.3 In vivo Assessment of Antifungal Activity

A smaller number of research groups have moved beyond the *in vitro* environment and are investigating the in vivo efficacy of those extracts that show promise i the laboratory. This is a more complex and costly activity which not only does the activity against the microorganisms need to be evaluated, but there must also be consideration of mammalian cell toxicity and allergic reactions. To date most in vivo testing of plant extracts has involved the use of essential oils against human skin infections, particularly fungal infections, and testing of extracts follow standard clinical trial protocols. Tea tree oil has been evaluated for use in athlete's foot with equivocal results, PolyToxinol (a mix of various essential oils) has shown promise against chronic methicillin-resistant *Staphylococcus aureus* (MRSA) osteomyelitis, and essential oil-containing mouthwashes have demonstrated efficacy against oral bacteria. It is important to note here that demonstrated activity in vitro does not always translate to activity in vivo. The best example of this is tea tree oil, which has been shown to have excellent activity in vitro against the fungi responsible for various types of ringworms (MIC 0.004–0.06 %) yet the results from clinical trials have been far from conclusive. This illustrates the caution with which researchers should view results from in vitro assays and reinforces the need for clinical trials of plant extracts that show therapeutic promise (Natarajan et al. 2001; Jenny 2006).

11.4 Antifungal Metabolites in Medicinal Plants

Since the plant kingdom provides a useful source of lead compounds of novel structure, a wide-scale investigation of species from the tropics has been considered. The common herbs tarragon and thyme both contain caffeic acid, a representative of a wide group of phenylpropane-derived compounds which is effective against fungi (Cole 1994). Various plant sources possessing antifungal metabolite are summarized in Table 11.2.

11.5 Major Groups of Antifungal Metabolites

Mostly essential oils are showing antifungal activity, for e.g., Ajoene, thymol, carvacrol, and linalool which are proved to be antifungal and tested against various fungal species such as *Sclerotinia sclerotiorum*, *Rhizopus stolonifer*, and *Mucor* sp. in a closed system. Plants have limitless ability to synthesize aromatic substances of different functional groups, Most of phenols and their oxygenated derivatives. Grapes contain several antifungal compounds (e.g. caffeic acid, chlorogenic acid, pterostilbene, resveratrol, and viniferin). These compounds were not likely idly generated. They probably evolved to protect the grape from its fungal enemies, Most plant effective constituents are secondary metabolites, out of which at least 13,000 have been isolated that is less than 10 % of the total (Schultes 1978). Plants are rich source of bioactive secondary metabolites of wide variety such as tannins, terpenoids, saponins, alkaloids, flavonoids, and other compounds, reported to have in vitro antifungal properties given in Table 11.3 (Arif et al. 2011).

A novel alkaloid, 2-(3,4-dimethyl-2,5-dihydro-1*H*-pyrrol-2-yl)-1-methylethyl pentanoate was isolated from the plant *Datura metel* showed *in vitro* as well as in vivo activity against *Aspergillus* and *Candida* species (Bahceevli et al. 2005). Another novel alkaloid, 6, 8-didec-(1*Z*)-enyl-5, 7-dimethyl-2, 3- dihydro-1H-indolizinium from *Aniba panurensis* demonstrated the activity against a drug-resistant strain of *C. albicans* (Ferheen et al. 2005). The antifungal alkaloids β-carboline, a tryptamine- and two phenyl ethylamine-derived alkaloids and *N*-methyl-*N*-formyl-4-hydroxy-beta-phenylethylamine from *Cyathobasis fruticulosa* and haloxylines A and B, new piperidine from *Haloxylon salicornicum* showed antifungal potentials (Singh et al. 2003). Cocsoline, a bisbenzylisoquinoline alkaloid from the *Epinetrum villosum* showed antifungal activities (Morteza et al. 2003). The alkaloids *N*-methylhydrasteine hydroxylactam and 1-methoxy -berberine chloride from *Corydalis longipes* showed high efficacy individually (Thouvenal et al. 2003). Four alkaloids, dicentrine, glaucine, protopine, and alpha-allocryptopin from *Glaucium oxylobum* exhibited good activity against *Microsporum gypseum*, *M. canis*, *T. mentagrophytes,* and *Epidermophyton floccosum* (Singh et al. 2000). From the root bark of *Dictamnus dasycarpus* two antifungal furoquinoline alkaloids were isolated. 3-methoxysampangine from *Cleistopholis patens* exhibited significant antifungal activity against *C. albicans*, *A. fumigatus*, and *C. neoformans* (Slobodmikova et al. 2004).

Table 11.2 Plant sources of antifungal metabolite (Khare 2007)

Plant source	Family	Antifungal metabolite
Agropyron repens Beauv	Gramineae (Poaceae)	Agropyrene
Alpinia officinarum Hance	Zingiberaceae	Flavones from rhizomes are strongly antifungal
Arachis hypogaea Linn	Papilionaceae; Fabaceae	Capric acid, obtained from the (red) skin, showed antifungal activity against *A. niger*
Avena sativa Linn	Gramineae; Poaceae	Avenacosides exhibit strong antifungal activity in vitro
Swartzia polyphylla	Fabaceae	95 % ethanol extract of bark afforded the flavonoids biochanin A and Dihydrobiochanin A as antifungal constituents
Echinops echinatus Roxb	Compositae; Asteraceae	Apigenin and its derivatives, echinacin and echinaticin show antifungal activity
Entada scandensauct. non-Benth. Synonym: *E. phaseoloides* Merrill. *E. pursaetha* DC. *Mimosa entada* Linn	Momosaceae	Entagenic acid, a sapogenin of entada saponin IV, imparts antifungal activity to the bark
Eriobotrya japonica Lindl	Rosaceae	Eriobofuran
Feronia limonia (Linn.) Swingle. Synonym: *F. elephantum* Corr	Rutaceae	Antifungal compounds, psoralene from stem bark; xanthotoxin and osthenol from root bark and 2,6-dimethoxybenzo-quinone from the fruit shell are reported
Fumaria vaillantii Loisel	Fumariaceae	Alkaloids, narlumidine and protopine, exhibit significant antifungal activity
Gardenia jasminoides Ellis. Synonym: *G. florida* Linn. *G. augusta* Merrill	Rubiaceae	The leaves contain an antifungal compound, cerbinal
Magnolia grandiflora Linn	Magnoliaceae	The sesquiterpene ketone, cyclocolorenone, also found in leaves, shows antifungal activity
Mirabilis jalapa Linn	Nyctaginaceae	Two Mirabilis jalapa antimicrobial proteins, MJ-AMP-1 and MJ-AMP-2, isolated from seeds, showed broad spectrum antifungal actvity involving a number of pathogenic fungi

(continued)

Table 11.2 (continued)

Plant source	Family	Antifungal metabolite
Myristica fragrans Houtt	Myristicaceae	The resorcinols, malabaricones B and C, isolated from the seed coat (mace) exhibited strong antibacterial and antifungal activities
Parmelia perlata (Huds.) Ach	Parmeliaceae	Several lichen species contain abundant quantities of usnic acid which exhibits antimicrobial and antifungal activity and is immunologically active in contact dermatitis
Polygonum hydropiper Linn	Polygonaceae	Polygodial and warburganal possess significant antifungal property. Warburganal also possesses potent cytotoxic and antibiotic activity. (The herb is used against cancer)
Polyscias fruticosa (L.) Harms. Synonym: *Nothopanax fruticosum* (L.) Miq. *Panax fruticosus* L	Araliaceae	The root contains polyacetylenes, falcarinol and heptadeca derivatives. Falcarinol and heptadeca exhibited strong antibacterial activity against Gram-positive bacteria and the dermatophytic bacteria, also showed antifungal activity
Prosopis chilensis Stuntz. Synonym: *Prosopis juliflora* DC	Mimosaceae	A mixture of alkaloids containing mainly juliprosine and *iso*juliprosine showed significant antifungal activity against dermatophytes (comparable to griseofulvin)
Ranunculus arvensis Linn	Ranunculaceae	The leaves contain the antifungal lactone protoanemonin which inhibited growth of *E. floccosum* and the yeast *Rhodotorula glutinis*
Sapindus mukorossi Gaertn	Sapindaceae	Saponin A and C sapindoside A and B, extracted from the fruit rind, showed antifungal activity
Sophora tomentosa Linn	Popilionaceae; Fabaceae	Sophoraisoflavone A exhibits antifungal activity
Trachyspermum ammi (Linn.) Sprague. Synonym: *T. copticum* Link. *Carum copticum* Benth. ex Hiern	Umbelliferae; Apiaceae	Thymol is a powerful antiseptic and antifungal
Ziziphus rugosa Lam	Rhamnaceaea	The cyclopeptide alkaloids of the plant show antibacterial as well as antifungal activity

Table 11.3 Various antifungal metabolites and their minimum inhibitory concentration (Arif et al. 2011)

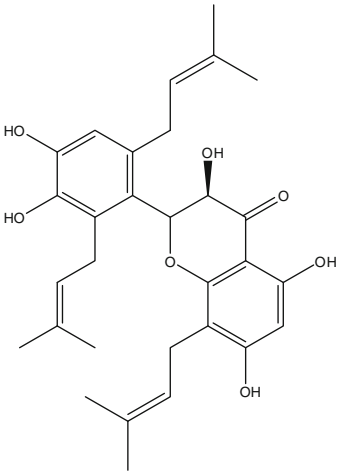
Class	Metabolite	Source	MIC	Fungal strains	References
Phenol	Eriosemanones A-D		5 mg/mL		Guang et al. (1995)
	Dihydro-*N*-caffeoyltyramine, *trans*-*N*feruloyloctopamine, trans-*N*-caffeoylt -yramine, and *cis*-*N*-caffeoyltyramine	*Lycium chinense*	5–10 μg/mL	*Candida albicans*	Lee et al. (2004)
	1-galloyl-*β*-D-glucopyranosyl-(1– > 4)-*β*-D-galactopyranoside, 2-methoxy-5-(1′,2′,3′-trihydroxypropyl)-phenyl-1-*O*-(6″-galloyl)-*β*-D-glucopyranoside and 2-methoxy-5-hydroxymethyl-phenyl-1- *O*-(6″-galloyl)-*β*-D-glucopyranoside	*Baseonema acuminatum*	25–100 μg/mL(IC 50)	*Candida albicans*, *T. mentagrophytes*, *T. rubrum*, *A. niger*	De Leo et al. (2004)
Flavonoids	Eupomatenoid-3, eupomatenoid-5, conocarpan and orientin	*Piper solmsianum*	2 to 60 μg/mL		De Campos et al. (2005)
	Flavonoids	*Inula viscosa*	0.01 mg/mL	*Dermatophytes*	Cafarchia et al. (2002)
Coumarins	Clausenidin, dentatin, nor-dentatin, and carbazole alkaloid, clauszoline J	*Clausena excavatea*	50 μg/mL	*T. mentagrophytes*, *T. rubrum*, *A. niger* and *C. albicans*	Sunthiktikawinsakul et al. (2003)
Quinones	Hopeanolin, an unusual resveratrol trimer with an O-quinone nucleus	*Hopea exalata*	0.1–22.5 μg/mL		Ge et al. (2006)
Saponins	Spirostanol saponins	*Smilax medica*	6.25–50 μg/mL	Human pathogenic yeasts *C. albicans*, *C. glabrata* and *C. tropicalis*	Sauton et al. (2006)
	Saponins	*Tribulus terrestris*	0.15 mg/ml	Fluconazole resistant *Candida* strains	Bedir et al. (2002)
	Tigogenin-3-*O*-*β*-D-glucopyranosyl (1– > 2)-[*β*-D-xylopyranosyl (1– > 3)]-*β*-D-glucopyranosyl (1– > 4)-*β*-D-galactopyranoside	*Tribulus terrestris*	4.4, 9.4 μg/mL, 10.7, 18.7 μg/mL, 8.8, 18.4 μg/mL	*Candida* species, *C. neoformans*, *C. krusei*	Zhang et al. (2005)

(continued)

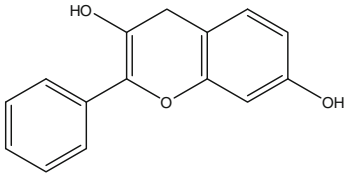
Table 11.3 (continued)

Class	Metabolite	Source	MIC	Fungal strains	References
Xanthones	Caledonixanthone E **(19)**	*Calophyllum Caledonicum*	MIC80 ¼ 8 mg/mL		Larcher et al. (2004)
	Soprenylated xanthones, toxyloxanthone C, and wighteone **(20)**	*Calophyllum Caledonicum*	25 and 12.5 mg/mL	*C. albicans*	Wang et al. (2005)
Terpenoids and essential oils	α − *cis*-ocimene,3,7-dimethyl-1,6-octadien-3-ol and ntransnerolidol	*Litsea cubeba*	0.03–0.4 μL/mL	*Pathogenic fungi*	Shin and Kang (2003)
	1′-acetoxychavicol acetate	*Alpinia galanga*	0.024 μg/mL	Fungal pathogens	Latha et al. (2009)
	Clove oil	*Eugenia caryophyllus*	0.051 mg/mL	*Candida albicans*	
Alkaloids	Jatrorrhizine	*Mahonia aquifolium*	62.5 to 125 μg/mL	All fungal species	Jung et al. (2006)

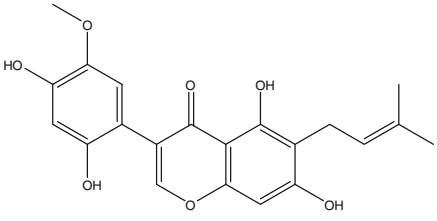
Flavonoids are hydroxylated phenolic substances synthesized by plants in response to microbial infection. Their activity is probably due to their ability to interact with extracellular and soluble proteins and with fungal cell walls. High lipophilic nature of flavonoids may also disrupt fungal membranes (Tsuchiya et al. 1996). Flavonoids isolated from the stem bark of *Erythrina burtii* were reported for antifungal activity (Yenesew et al. 2005). 4-methoxy-5,7-dihydroxyflavone 6-C-glucoside (isocytisoside) from the leaves and stems of *Aquilegia vulgaris* showed activity against the mold *A. niger* (Bylka et al. 2004). Pelalostemumol **(6)** from *Pelalostemium* had strong antifungal activity against many pathogenic fungi (Hufford et al. 1993). Galangin **(7)** derived from the perennial herb *Helichrysum aureonitens*, seems to be a particularly useful compound, since it has shown activity against wide range of fungi (Afolayan and Meyer 1997). A flavonoid from rhizome of *Alpinia officinarum* had strong antifungal activity against different pathogenic fungi (Ray and Majumdar 1975, 1976). Minimum inhibitory concentration (MIC) of the identified flavonoid against the fungi was determined as 3 μg/mL. A flavon 3,4',5,7-tetraacetyl quercetin isolated from heartwood of *Adina cordifolia* exhibited moderate antifungal activity against *A. fumigatus* and *C. neoformans* (Rao et al. 2002). Flavonoid derivative phloretin from *Malus sylvestris* have antifungal properties (Hunter et al. 1993). Isopiscerythrone **(8)**, allolicoisoflavone A **(9)**, piscisoflavones A and B from different plants were reported to be endowed with antifungal activity (Moriyama et al. 1992).



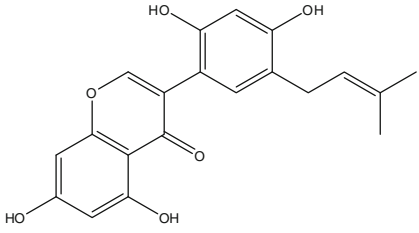
Pelalostemumol (6)



Galangin (7)

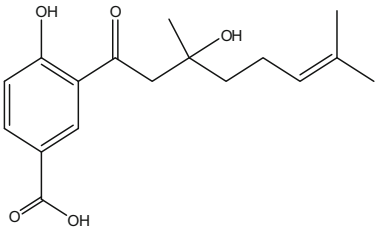


Isopiscerythrone (8)

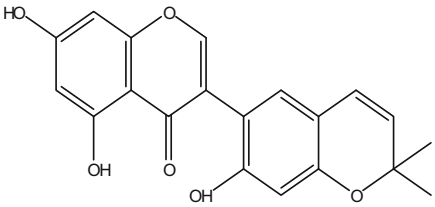


Allolicoisoflavone (9)

Increased hydroxylation results in increased toxicity and more highly oxidized phenols have more antifungal activities (Mason and Wasserman 1987; Thornes et al. 1997). The mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, reaction with sulfhydryl groups or nonspecific interactions with the proteins. Tannins and salicylic acid are polyphenol compounds extracted from *Gaullher procumbens*, *Rhammus purshiana,* and *Anacardium pulsatilla* showed antifungal activity (Thornes et al. 1997; Otshudi et al. 2005). *Piper crassinervium*, *P. aduncum*, *P. hostmannianum,* and *P. gaudichaudianum*, contain phenolic acid derivatives crassinervic acid **(10)**, aduncumene, hostmaniane, and gaudichaudanic acid, respectively, were reported for fungitoxic activity (Lago et al. 2004). A phenolic compound from *Croton hutchinsonianus*, and pinosylvin **(11)**, a constituent of pine, showed growth inhibitory activity against *C. albicans* and *Saccharomyces cerevisiae* (Guang et al. 1995; Lee et al. 2005;Athikomkulchal et al. 2006).

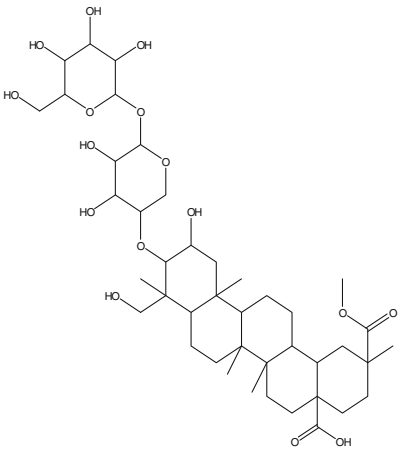


Crassinervic acid (10)

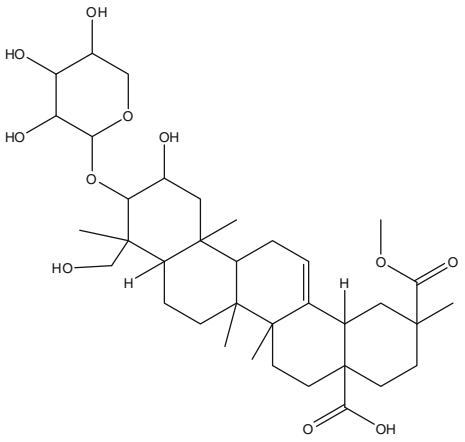


Pinosylvin (11)

Saponins are stored in plant cells as inactive precursors but are readily converted into biological active antibiotics by enzymes in response to pathogen attack. Saponins are glycosylated compounds widely distributed in plant kingdom and can be divided into three major groups, a triterpenoid, a steroid, or a steroidal glycoalkaloid. CAY-1, a triterpene saponin from the *Capsicum frutescens* was found to be active against 16 different fungal strains, including *Candida* spp., *A. fumigatus* and *C. neoformans* (Renault et al. 2003). Importantly, CAY-1 appears to act by disrupting the membrane integrity of fungal cells. *Mollugo pentaphylla*, a tropical herb, contains an antifungal saponin, mollugogenol-A. Phytolaccosides B **(12)** and E **(13)** from *Phytolacca tetramera* showed antifungal activities against a panel of human pathogenic opportunistic fungi. Two saponins from the stems of *Anomospermum grandifolium* jujubogenin 3-*O*-α-l-arabinofuranosyl(1–>2)- [*β*-D-glucopyra -nosyl(1–>6)*β*-D-glucopyranosyl(1–>3)]-α-l-arabinopyranoside, ujubogenin 3-*O*-α-l-arabinofuranosyl(1–>2)-[6-*O*-[3-hydroxy-3-methylglutaryl]- *β*-D-glucopyranosyl(1–>3)]-α-l-arabinopyranoside and a new lupane saponin, 3*β*-hydroxylup-20(29)-en-27,28-dioic acid 28-*O*-*β*-D-glucopyranosyl(1–>2)-[*β*- D-xylopyranosyl(1–>3)]-*β*-D-xylopyranosyl(1–>2)-*β*-D-glucopyranoside ester, jujubogenin 3-*O*-α-l-arabinofuranosyl(1–>2)-[*β*-D-glucopyranosyl (1–>3)]-α-larabinopyranoside, and 3*β*-hydroxylup-20(29)-ene-27,28-dioic acid revealed antifungal properties against *C. albicans* (Du et al. 2003).

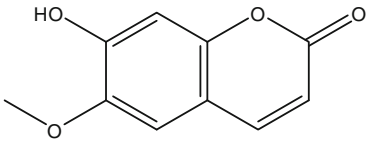


Phytolaccosides B(12)

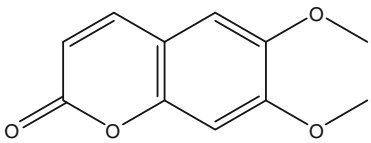


Phytolaccosides E (13)

Coumarins have been reported to stimulate macrophages which could have an indirect negative effect on infections. Coumarins are phenolic substances made of fused benzene and α-pyrone rings. Hydroxycoumarin, scopoletin **(14)** was isolated from seed kernels of *M. azedarach* reported to be antifungal against *Fusarium verticillioides* (Carpinella et al. 2005). Phytoalexins, which are hydroxylated derivatives of coumarins, are produced in carrots in response to fungal infection and can be presumed to have antifungal activity (Hoult and Paya 1996). Tithoniamarin is a new isocoumarin dimer isolated from *Tithonia diversifolia* showed antifungal and herbicidal activities (Yemele et al. 2006). A coumarin namely, 6,7-dimethoxy - coumarin **(15)**, isolated from *P. digitatum*-infected Valencia fruit confers resistance against the mycotoxigenic fungi *A. parasiticus* (Mohanlall et al. 2006).



Scopoletin (14)

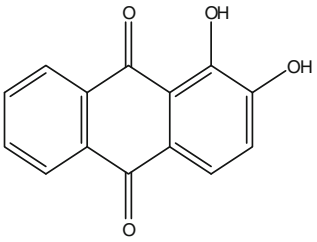


6,7- dimethoxycoumarin (15)

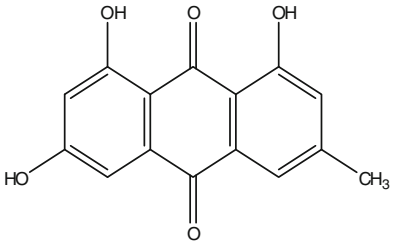
The essential oils are secondary metabolites that are highly enriched in compounds based on isoprene structure. They are called terpenes, their general chemical structure is $C_{10}H_{16}$, and they occur as diterpenes, triterpenes, and tetraterpenes (C20, C30, and C40), as well as hemiterpenes (C5) and sesquiterpenes (C15). The mechanism of action of terpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic nature. Mendoza et al. (1997) found that increasing the hydrophilicity of kaurene diterpenoids by addition

of a methyl group drastically reduced their antimicrobial activity. The antifungal activities of the essential oil from *Agastache rugosa* and its main component, estragole, combined with ketoconazole, were reported to show significant synergistic effects (Shin 2004). The roots of *Delphinium denudatum* have yielded 8-acetylheterophyllisine, panicutine, and 3-hydroxy-2-methyl-4*H*-pyran-4- one has shown antifungal activity against a number of human pathogenic fungi (Ahmed et al. 2004). From other *Centaurea* species, *C. thessala* and *C. attica*, two eudesmanolides, 4-*epi*-sonchucarpolide, 8-(3-hydroxy-4-acetoxy-2-methylene-butanoyloxy) derivative, and eudesmane derivative named atticin showed a considerable antifungal effect against nine fungal species (Srinivas et al. 2006). A-mesterol, isolated from *Amaranthus viridis* strongly inhibited the growth of pathogenic fungus (Tripathi et al. 1978). *A. sativum* oil exhibited the growth of *T. rubrum*, *T. erinacei*, and *T. soudanense* with MIC of 4.0 μg/mL, while the activities of *A. cepa* and *A. fistulosum* were relatively mild (D'Auria et al. 2005). A fruit pulp extract of *Detarium microcarpum* endowed with four new clerodane diterpenes which showed antifungal activity (Cavin et al. 2006). The diterpenoids 16α-hydroxy-cleroda-3,13-(14)-*Z*-diene-15,16-olide and 16-oxo-cleroda-3,13-(14)-*E*-diene-15-oic acid isolated from the hexane extract of the seeds of *Polyalthia longifolia* demonstrated significant antifungal activity (Marthanda et al. 2005). Five new diterpenoids from *Casimirella* namely, humirianthone, 1-hydroxy-humirianthone, 15*R*-humirianthol, patagonol and showed activity against pathogenic fungi. The triterpenoids pristimerin and celastrol isolated from the roots of *Celastrus hypoleucus* exhibited inhibitory effects against diverse pathogenic fungi (Adou et al. 2005). Carvone, dinydrocarvone, limonene, dillapiole, and dillapional from *Anethum sowa* revealed antifungal activity at a concentration of 1:100 and 1:250 (Saksena et al. 1984; Shankaracharya et al. 2000; Tostao et al. 2005). A derivative of dillapiole, isodilapiole tribromide found to more active (Saxena et al. 1990). An Indian chemotype *Ocimum gratissimum*, with a high level of ethyl cinnamate, showed, in vitro, an interesting spectrum of antifungal properties (Atta-ur-Rahman and Choudhary 1995).

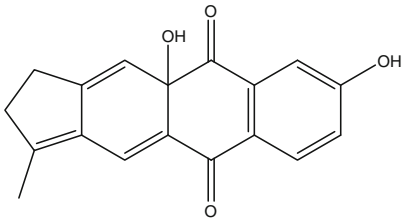
Quinones are aromatic rings with two ketone substitutions and characteristically highly reactive. They can switch between diphenol (or hydroquinone) and diketone (or quinone) easily through oxidation and reduction reactions. These compounds, being colored, are responsible for the browning reaction in cut or injured fruits and vegetables. In addition to providing a source of stable free radicals, quinones are known to complex irreversibly with nucleophilic amino acids in proteins (Stern et al. 1996). Therefore the quinone inactivates the protein and impairs their function. Quinones bind with surface-exposed adhesins, cell wall polypeptides, membrane-bound enzymes and form complex which inactivate the enzymes. Alizarin **(16)** and emodin **(17)** of *Rubia tinctorum* and *Rhamnus frangula* have antifungal activity. The naphthoquinones kigelinone **(18)**, isopinnatal, dehydro-alpha- lapachone, and lapachol from *Kigelia pinnata* were reported for antifungal activity (Singh et al. 1986).



Alizarin (16)

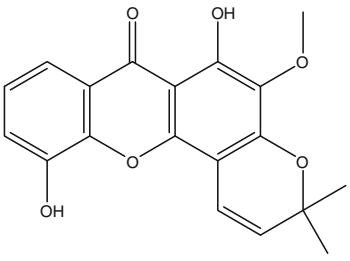


Emodin (17)

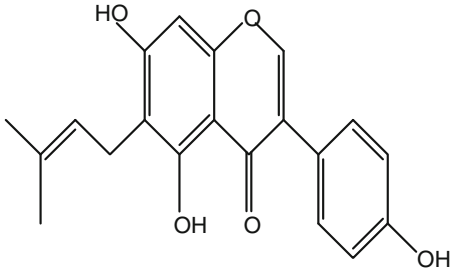


Kigelinone (18)

Xanthones are a restricted group of plant polyphenols, biosynthetically related to the flavonoids. These are planar-six carbon molecules in a conjugated ring system consisting of a backbone molecule and various chemical groups attached to it. Xanthone backbone consists of two benzene rings attached through a carbonyl group and oxygen not allowing free rotation about the carbon bonds. The unique backbone along with type and position of the attached chemical groups defines specific properties of xanthones. Xanthones possess numerous bioactive capability including antifungal properties (Mendoza et al. 1997). Xanthones from the green fruits of *Garcinia mangostana* were reported to have strong antifungal activities (Dhamaratne et al. 2005).



Caledonixanthone E(19)



Wighteone(20)

Recent interest has been focused mostly on studying fungi by these macromolecules, such as that from the herbaceous *Amaranthus*, has long been known (Mendez et al. 1990). Thionins are peptides commonly found in barley and wheat and consist of 47 amino acid residues. Thionins AX1 and AX2 from sugar beet were active against fungi but not bacteria (Kragh et al.1995; Ankri and Mirelman 1999). A novel lectin was isolated from the roots of *Astragalus mongholicus* and a protein with a novel *N*-terminal sequence from *Ginger* rhizomes exerted antifungal activity toward various fungi (Ngai et al.2005). An antifungal peptide was reported from fresh fruiting bodies of the mushroom *Agrocybe cylindracea* (Ye et al. 2001). Two antifungal peptides from seeds of *Pharbitis nil* exhibited potent antifungal activity against fungi. Concentrations required for 50 % inhibition of fungal growth were ranged from 3 to 26 μg/mL and from 0.6 to 75 μg/mL (Wang and Ng 2005). A novel antifungal peptide, cucurmoschin, was isolated from the seeds of the black pumpkin inhibited mycelial growth in the fungi (Ye and Ng 2002). A peptide designated cicerarin from seeds of the green chickpea *Cicer arietinum* showed antifungal activity. The antifungal activity was preserved after exposure to 100 °C for 15 min (Ye et al. 2002). Two other antifungal peptides cicerin and arietin were reported from seeds of the chickpea (*C. arietinum*). Arietin manifested a higher antifungal potency toward *Mycosphaerella arachidicola*, *Fusarium oxysporum* and *Botrytis cinerea* (Wang and Ng 2001). An antifungal peptide designated angularin isolated from the adzuki bean was shown antifungal activity against a variety of fungal species (Park et al. 2005). Two novel antifungal peptides, designated alpha- and beta-basrubrins, respectively, were isolated from seeds of the Ceylon spinach *Basella rubra* (Taira et al. 2005). An antifungal protein, AFP-J, was purified from potato tubers, *Solanum tuberosum* strongly inhibited the growth of yeasts *C. albicans*, *Trichosporon beigelii* and *S. cerevisiae* (Shenoy et al. 2006). Pineapple leaf chitinase-B from *Ananas comosus* exhibits strong antifungal activity toward *Trichoderma viride* (Yadav et al. 2007). Another chitinase with antifungal activity was also purified from the bulbs of the plant *Urginea indica*, known as Indian squill.

11.6 Importance of Antifungal Agents

Antifungal compounds are investigated in plant extracts so that they can assist in protecting cultivated plants from fungal attack. Resin is secreted in response to physical wounding or attack by fungal pathogens and insects such as bark beetles. Resins, however, are not all related to gum, which may have the same function in other species. This defense reaction by conifers is adaptively important to the survival of conifers in natural habitats, because the oleoresins are antifungal and toxic to bark beetles. Wounded areas in the bark of a tree trunk or branch physically become sealed by the solidification of the resin acids after the turpentine has evaporated.

Antifungal metabolites help in storing of extracts. It was expected that dried extracts would be very stable. But surprisingly the acetone extracts of the combretaceae retained antibacterial and anti-inflammatory activity over prolonged periods even when stored in a dissolved state at room temperature. This may be possible due to antibacterial and antifungal activity of these compounds (Eloff et al. 2001).

11.7 Conclusions

These successes emphasize the potential value of newly developed plant products. The emergence of drug resistance in pathogenic fungi as well as bacteria and the non availability of suitable antifungal drugs for certain infections such as systemic and mucosal mycoses (e.g., Vaginal candidiasis) points out the urgent need for the discovery and development of alternative drugs from natural sources. Biological resources such as plants hold a great promise as source of curative molecules. However these need thorough screening for their scientific and large-scale use as drugs.

Single and poly herbal preparations have been used throughout history for treatment of various types of illnesses. Ayurvedic drugs have been Proven Promoting in inhibiting microbes that have developed drug resistance. The ultimate conclusion of this study supports the ayurvedic medicines use of different plant in treating different infections conserved by pathogenic fungal inflation by using a single or combined formulation. It also suggested that a great alternation should be paid to medicinal plants which are found to have plant of pharmacological art uses. That could be better when considering a natural forced and feed additional to improve human and animal health.

11.8 Future Prospects

A large number of metabolites from plants have been screened for their antifungal activity. A large variety of formulations already exist for intravaginal therapy (tablets, creams, suppositories, pessaries, foams, solutions, ointments, and gels). However, their efficiency is often hindered by poor retention at the site of action due to the self-cleansing action of the vaginal tract. Thus we can look forward for formulating the antifungal metabolite into a modernized formulation. Liposomes offer an effective delivery option because of their ability to prolong contact of drug with the mucosal surface without inducing adverse local effects on the epithelium. The therapeutic potential of any drug depends on its bioavailability, retention time, and amount of drug at target site. One experiment showed that incorporating clove oil into liposomes can considerably increase its therapeutic efficacy. Although the MIC of clove oil suggests it to be less potent than antifungal drugs such as

nystatin, it can be safely said that the fungicidal potency of a liposomal formulation of the oil compares very well with that of nystatin, while providing for a less toxic, safe, and inexpensive alternative to commercial drugs without the risk of ever-increasing resistance shown by the target pathogens, toxicity problems at the increasing required doses, and side-effects.

References

Adou E, Williams RB, Schilling JK, Malone S, Meyer J, Wisse JH, Frederik D, Koere D, Werkhoven MC, Snipes CE, Werk TL, Kingston DG (2005) Cytotoxic diterpenoids from two lianas from the Suriname rainforest. Bioorg Med Chem 13:6009–6014

Afolayan AJ, Meyer JJ (1997) The antimicrobial activity of 3,5,7-trihydroxyflavone isolated from the shoots of *Helichrysum aureonitens*. J Ethnopharmacol 57:177–181

Ahmed AA, Bishr MM, El-Shanawany MA, Attia EZ, Ross SA (2004) Rare trisubstituted sesquiterpenes daucanes from the wild Daucus carota. Phytochemistry 66:1680–1684

Aiyegoro OA, Afolayan AJ, Okoh AI (2008) *In vitro* time kill assessment of crude methanol extract of *Helichrysum pedunculatum* leaves. Afr J Biotechnol 7(11):1684–1688

Ankri S, Mirelman D (1999) Antimicrobial properties of allicin from garlic. Microbes Infect 1:125–129

Antonov A, Stewart A, Walter M (1997) Inhibition of conidium germination and mycelial growth of *Botrytis cinerea* by natural products. In: 50th Conference proceeding of the New Zealand plant protection society, pp 159–164

Arif T, Mandal TK, Dabur R (2011) Natural products: antifungal agents derived from plants. Opportunity challenge and scope of natural products in medicinal chemistry. pp 283–311

Ateb DA, ErdoUrul OT (2003) Antimicrobial activities of various medicinal and commercial plant extracts. Turk J Biol 27:157–162

Athikomkulchal S, Prawat H, Thasana N, Ruangrungsi N, Ruchirawat S (2006) COX-1, COX-2 inhibitors and antifungal agents from *Croton hutchinsonianus*. Chem Pharm Bull 54:262–264

Atta-ur-Rahman MI, Choudhary D (1995) Diterpenoid and steroidal alkaloids. Nat Prod Rep 12:361–379

Awasthi AK, Bisht GS, Anroop B (2005) *In vitro* antimicrobial activity of *Swertia chirata*. Indian J Nat Prod 21:27–29

Bahceevli AK, Kurnan S, Kolak U, Topcu G, Adou P, Kingston DG (2005) Alkaloids and aromatics of *Cyathobasis fruticulosa* (Bunge) Aellen. J Nat Prod 8:956–958

Bedir E, Khan IA, Walker LA (2002) Biologically active steroidal glycosides from *Tribulus terrestris*. Pharmazie 57:491–493

Bobbarala V, Katikala PK, Naidu KC, Penumajji S (2009) Antifungal activity of selected plant extracts against phytopathogenic fungi *Aspergillus niger* F2723. Indian J Sci Technol 2:87–90

Bylka W, Szaufer M, Matlawska J, Goslinska O (2004) Antimicrobial activity of isocytisoside and extracts of *Aquilegia vulgaris* L. Lett Appl Microbiol 39:93–97

Carpinella MC, Ferravoli CG, Palacios SM (2005) Antifungal synergistic effect of scopoletin, a hydroxycoumarin isolated from *Melia azedarach* L. fruits. J Agric Food Chem 53:2922–2927

Cavin AL, Hay AE, Marston A, Stoeckli-Evans H, Scopelliti R, Diallo D, Hostettmann K (2006) Bioactive diterpenes from the fruits of *Detarium microcarpum*. J Nat Prod 69:768–773

Chen LC, Blank ES, Casadevall A (1996) Extracellular proteinase activity of *Cryptococcus neoformans*. Clin Diagn Lab Immunol 3:570–574

Chowdhury JU, Yusuf M, Begum J, Taniya AJ, Hossain ME, Hossain MA (2005) (BCSIR, Laboratories, PO Chittagong Cantonment, Chittagong-4220, Bangladesh) Aromatic plants of Bangladesh: composition and antimicrobial activities of leaf oil from *Curcuma longa* Linn. Indian Perfumer 49(1):61–65

Cole MD (1994) Key antifungal and antibacterial assays, a critical review. Biochem Syst Ecol 22:837–856

CSIR (1998) Wealth of India, publications and information directory New Delhi. CSIR, India, p 164

Dabur R, Gupta A, Mandal TK, Singh DD, Bajpai V, Gurav AM, Lavekar GS (2007) Antimicrobial activity of some Indian medicinal plants. Afr J Trad Cam 4(3):313–318

D'Auria FD, Tecca M, Strippoli V, Salvatore G, Battinelli L, Mazzanti G (2005) Antifungal activity of *Lavandula angustifolia* essential oil against *Candida albicans* yeast and mycelial form. Med Mycol 43:391–396

De Cafarchia C, Laurentis N, Milillo MA, Losacco V, Puccini V (2002) Antifungal activity of essential oils from leaves and flowers of *Inula viscosa* (Asteraceae) by Apulian region. Parasitologia 44:153–156

De Campos MP, Chechinel Filho V, Da Silva RZ, Yunes RA, Zacchino S, Juarez S, Bella Cruz RC, Cruz Bella (2005) A Evaluation of antifungal activity of *Piper solmsianum* C DC var. *solmsianum* (Piperaceae). Biol Pharm Bull 28:1527–1530

De Leo M, Braca A, De Tommasi N, Norscia I, Morelli I, BattinelliL L, Mazzanti G (2004) Phenolic compounds from *Baseonema acuminatum* leaves: isolation and antimicrobial activity. Planta Med 70:841–846

Dellar JE, Cole MD, Gray AI, Gibbons S, Waterman PG (1994) Antimicrobial sesquiterpenes from *Prostanthera affmelissifilia* and *P rotundifolia*. Phytochemistry 36:957–960

Deshpande S, Shah T, Shah G, Parmar NS (2005) A preliminary study on antimicrobial activity of *T. purpurpea*. Indian J Nat Prod 21:33–34

Dharmaratne HRW, Piyasena KGNP, Tennakoon SB (2005) A geranylated biphenyl derivative from *Garcinia malvgostana*. Nat Prod Res 19:239–243

Diba K, Gerami Shoar M, Shabatkhori M, Khorshivand Z (2011) Anti fungal activity of alcoholic extract of *Peganum harmala* seeds. J Med Plants Res 5:5550–5554

Duraipandiyan V, Ignacimuthu S (2011) Antifungal activity of traditional medicinal plants from Tamil Nadu. India Asian Pacific J Trop Biomed 1:S204–S215

Du Z, Zhu N, Ze-Ren-Wang-Mu N, Shen Y (2003) Two new antifungal saponins from the Tibetan herbal medicine Clematis tangutica. Planta Med 69:547–51

Edris AE, Farrag ES (2003) Antifungal activity of peppermint and sweet basil essential oils and their major aroma constituents on some plant pathogenic fungi from the vapor phase. Nahrung (Food) 2:117–121

Elisabetsky E, Souza GC (2002) Etnofarmacologia como ferramenta na busca de substâncias ativas In: CMO Simões, Farmacognosia da planta ao medicamento, UFRG e UFSC, Porto Alegre/Florianópolis, 107–122

Eloff JN, Jager AK, Van Staden J (2001) The stability and relationship between anti-inflammatory activity and antibacterial activity of southern African *Combretum* species. S Afr J Sci 97:291–293

Ferheen S, Ahmed E, Afza N, Malik A, Shah MR, Nawaz SA, Choudhary M (2005) Haloxylines A and B, antifungal and cholinesterase inhibiting piperidine alkaloids from *Haloxylon salicornicum*. Chem Pharm Bull 53:570–572

Fortes TO, Alviano DS, Tupinamba G, Padron TS, Antoniolli AR, Alviano CS, Seldin L (2008) Production of an antimicrobial substance against *Cryptococcus neoformans* by *Paenibacillus brasilensis* Sa3 isolated from the rhizosphere of *Kalanchoe brasiliensis*. Microbiol Res 163:200–207

Fostel J, Lartey P (2000) Emerging novel antifungal agents. Drug Discov Today 5:25–32

Franco J, Nakashima T, Franco L, Boller C (2005) Chemical composition and antimicrobial in vitro activity of the essential oil *Eucalyptus cinerea* F. Mull. ex Benth., Myrtaceae, extracted in different time intervals. Braz J Pharmacog 15:191–194

Ge HM, Huang B, Tan SH, da Shi H, Song YC, Tan RX (2006) Bioactive oligostilbenoids from the stem bark of *Hopea exalata*. J Nat Prod 69:1800–1802

Giordani R, Trebaux J, Masi M, Regli P (2001) Enhanced antifungal activity of ketoconazole by *Euphorbia characias* latex against *Candida albicans*. J Ethnopharmacol 78:1–5

Guang W, Fuzzati N, Li QS, Yang CR, Evans HS, Hostettmann K (1995) Polyphenols from *Eriosema tuberosum*. Phytochemistry 39:1049–1061

Hoult JRS, Paya M (1996) Pharmacological and biochemical actions of simple coumarins: natural products with therapeutic potential. Gen Pharmacol 27:713–722

Hostettmann K, Marston A (1994) Search for new antifungal compounds from higher plants. Pure Appl Chem 66:2231–2234

Hufford CD, Jia Y, Croom EM, Muhammed I, Okunade AI, Clark AM, Rogers RD (1993) Antimicrobial compounds from *Petalostemum purpureum*. J Nat Product 56:1878–1889

Hunter MD and Hull LA (1993) Variation in concentrations of phloridzin and phloretin in apple foliage.Phytochemistry 34: 1251–1254

Ibrahim MB (1997) Anti-microbial effects of extract leaf, stem and root bark of *Anogeissus leiocarpus* on *Staphylococcus aureaus*, *Streptococcus pyogenes*, *Escherichia coli* and *Proteus vulgaris*. J Pharma Devpt 2:20–30

Inouye S, Tsuruoka T, Uchida K, Yamaguchi H (2001a) Effect of sealing and Tween 80 on the antifungal susceptibility testing of essential oils. Microbiol Immunol 43:201–208

Inouye S, Uchida K, Yamaguchi H (2001b) *In vitro* and in vivo anti-*Trichophyton* activity of essential oils by vapor contact. Mycoses 44:99–107

Inouye S, Uchida K, Yamaguchi H, Miyara T, Gomi S, Amano M (2001c) Volatile aroma constituents of three labiateae herbs growing wild in the Karakoram-Himalaya district and their antifungal activity by vapor contact. J Essent Oil Res 13:69–72

Jansen AM, Cheffer JJC, Svendsen AB (1987) Antimicrobial activity of essential oils: a 1976–1986 literature review. Aspects of the test methods. Planta Med 40:395–398

Jeevan Ram A, Bhakshu LM, Venkata Raju RR (2004) *In vitro* antimicrobial activity of certain medicinal plants from Eastern Ghats, India, used for skin diseases. J Ethnopharmacol 90:353–357

Jenny MW (2006) Modern Phytomedicine; turning medicinal plants into drugs In: Iqbal A, Farrukh A, Owais M (eds) Methods for testing the antimicrobial activity of extracts, antifungal assay. Wiley-Vch, pp 157–172

Jung HJ, Sung WS, Yeo SH, Kim HS, Lee IS, Woo ER, Lee DG (2006) Antifungal effect of amentoflavone derived from *Selaginella tamariscina*. Arch Pharm Res 29:746–751

Kavya R, Shrungashree RM and Suchitra SV (2007) Evaluation of antimicrobial activity of Cow urine distillate and plant extracts, Project Dissertation, Kuvempu University, Shankaraghatta, Shivammogga, Karnataka, India

Khare CP (2007) Indian medicinal plants: an illustrated dictionary, Springer 27–737

Khattak S, Rehman S, Shah HU, Ahmad W and Ahmad M (2005) Biological effect of indigenous medicinal plants *Curcuma longa* and *Alpina galangal*. Fitoterapia 76: 254–257

Kragh KM, Nielsen JE, Nielsen KK, Dreboldt S, Mikkelsen JD (1995) Characterization and localization of new antifungal cysteine-rich proteins from *Beta vulgaris*. Mol Plant-Microbe Interact 8:424–434

Kubo I, Muroi H, Himejima M (1993) Combination effects of antifungal nagilactones against *Candida albicans* and two other fungi with phenylpropanoids. J Nat Prod 56:220–226

Kulkarni PH (2012) Anti microbial Plants of Ayurveda. http://www.drsharduli.com/journal/review/56/review articles

Kumar V, Shah T, Parmar NS, Shah GB, Goyal KR (2004) Antimicrobial activity of Peganum harmala. Indian J Nat Prod 21:24–26

Kurian P, Thomas M (2004) Neem oil- A preventive against leptospiral infection in man. Kottayam Kerala Aryavaidyan 18:41–44

Lago JH, Ramos CS, Casanova DC, Morandim AA, Bergamo DC, Cavalheiro AJ, Bolzani VS, Furlan M, Guimaraes EF, Young MC, Kato MJ (2004) Comparison of fascaplysin and related alkaloids: a study of structures, cytotoxicities, and sources. J Nat Prod 67:783

Larcher G, More C, Tronchin G, Landreau A, Seraphin D, Richommeand P, Bouchara JP (2004) Investigation of the antifungal activity of caledonixanthone E and other xanthones against *Aspergillus fumigatus*. Planta Med 70:569–571

Latha C, Shriram VD, Jahagirdar SS, Dhakephalkar PK, Rojatkar SR (2009) Antiplasmid activity of 1′-acetoxychavicol acetate from *Alpinia galanga* against multi-drug resistant bacteria. J Ethnopharmacol 123:522–525

Lee DG, Park Y, Kim MR, Jung HJ, Seu YB, Hahm KS, Woo ER (2004) Anti-fungal effects of phenolic amides isolated from the root bark of *Lycium chenense*. Biotechnol Lett 26:1125–1130

Lee SK, Lee HJ, Min HY, Park EJ, Lee KM, Ahn YH, Cho YJ, Pyee JH (2005) Antibacterial and antifungal activity of pinosylvin, a constituent of pine. Fitoterapia 76:258–260

Liu XT, Shi Y, Liang JY, Min ZD (2009) Antibacterial ent-rosane and ent-kaurane diterpenoids from *Sagittaria trifolia* var. *sinensis*. Chin J Nat Med 7(6):341–345

Mahesh B, Satish S (2008) Antimicrobial activity of some important medicinal plant against plant and human pathogens. World J Agri Sci 4:839–843

Mann A, Banso A, Clifford LC (2008) An antifungal property of crude plant extracts from *Anogeissus leiocarpus* and *Terminalia avicennioides*. Tanzania J Health Res 10:34–38

Marthanda M, Subramanyan M, Hima M, Annapurna (2005) Antimicrobial activity of clerodane diterpenoids from *Polyalthia longifolia* seeds. Fitoterapia 76:336

Mason TL, Wasserman BP (1987) Inactivation of red beet beta-glucan synthase by native and oxidized phenolic compounds. Phytochemistry 26:2197

Mendez E, Moreno A, Colilla F, Pelaez F, Limas GG, Mendez R, Soriano F, Salinas M, De Haro C (1990) Primary structure and inhibition of protein synthesis in eukaryotic cell-free system of a novel thionin, gamma-hordothionin, from barley endosperm. Eur J Biochem 194:533–539

Mendoza L, Wilkens M, Urzua A (1997) Antimicrobial study of the resinous exudate and diterpenoids and flavonoids isolated from some Chilean *Pseudognaphalium* (Asteraceae). J Ethnopharmacol 58:85

Mohanlall V, Odhav B (2006) Biocontrol of Aflatoxins B_1, B_2, G_1, G_2, and fumonisin B_1 with 6, 7-dimethoxycoumarin, a phytoalexin from *Citrus sinensis*. J Food Prot 69: 2224–2229

Moriyama M, Tahara S, Ingham JL, Mizutani J (1992) Isoflavones from the root bark of *Piscidia erythrina*. Phytochemistry 31: 683–687

Morteza-Semnani K, Amin G, Shidfar MR, Hadizadeh H, Shafiee A (2003) Antifungal activity of the methanolic extract and alkaloids of *Glaucium oxylobum*. Fitoterapia 74:493

Nair R, Chanda S (2005) Anticandidal activity of *Punica granatum* exhibited in different solvents. Pharm Biol 43:21–25

Natarajan S, Williamson D, Grey J, Harding KG, Cooper RA (2001) Healing of an MRSA-colonized, hydroxyurea-induced leg ulcer with honey. J Dermatol Treat 12:13–36

Ngai PH, Zhao Z, Ng TB (2005) Agrocybin, an antifungal peptide from the edible mushroom *Agrocybe cylindracea*. Peptides 26:191

Ogundipe O, Akinbiyi O, Moody JO (1998) Antibacterial activities of essential ornamental plants. Niger J Nat Prod Med 2:46–47

Otshudi AL, Apers S, Pieters L, Claeys M, Pannecouque C, De Clercq E, Van Zeebroeck A, Lauwers S, Frederich M, Foriers A (2005) Biologically active bisbenzylisoquinoline alkaloids from the root bark of *Epinetrum villosum*. J Ethnopharmacol 102:89–94

Park Y, Choi BH, Kwak JS, Kang CW, Lim HT, Cheong HS, Hahm KS (2005) Kunitz-type serine protease inhibitor from potato (*Solanum tuberosum* L cv Jopung). J Agric Food Chem 53:6491–6496

Perumal G, Subramanyam C, Natarajan D, Srinivasan K, Mohanasundari C, Prabakar K (2004) Antifungal activities of traditional medicinal plant extracts- A preliminary survey. J Phytolog Res 17:81–83

Portillo A, Vila R, Freixa B, Adzet T, Canigueral S (2001) Antifungal activity of Paraguayan plants used in traditional medicine. J Ethnopharmacol 76:93–98

Prashith KTR, Kavya R, Shrungashree RM, Suchitra SV (2010) Screening of selected single and polyherbal Ayurvedic medicines for antibacterial and antifungal activity. Ancient Sci Life 29:22–25

Rahalison L, Hamburger M, Hostettmann K, Monod M, Frenk E (1991) A bioautographic agar overlay method for the detection of antifungal compounds from higher plants. Phytochem Anal 2:199–203

Rao MS, Duddeck H, Dembinski R (2002) Isolation and structural elucidation of 3,4',5,7-tetraacetyl quercetin from *Adina cordifolia* (Karam ki Gaach). Fitoterapia 73:353–355

Ray PG, Majumdar SK (1975) New antifungal substance from *Alpinia officinarum*. Ind J Exp Biol 13:489–492

Ray PG, Majumdar SK (1976) Antifungal flavonoid from *Alpinia officinarum*. Ind J Exp Biol 14:712–714

Reddy PS, Jamil K, Madhusudhan P (2001) Antibacterial activity of isolates from *Piper longum* and *Taxus baccata*. Pharma Biol 39:236–238

Renault S, De Lucca AJ, Bove S, Bland JM, Vigo CB, Selitremikoff CP (2003) CAY-1, a novel antifungal compound from *Cayenne pepper*. Med Mycol 41:75–82

Resende JCP, Resende MA (1999) *In vitro* antifungal susceptibility of clinical isolates of *Candida* spp. from hospitalized patients. Mycoses 42:641–644

Rojas R, Bustamante B, Bauer J (2003) Antimicrobial activity of selected Peruvian medicinal plants. J Ethnopharmacol 88:199–204

Saksena S (1984) Enhancement in the antifungal activity of some essential oils in combination against some dermatophytes. Indian Perfumer 28 : 42–45

Samie A, Obi CL, Bessong PO, Namrita L (2005) Activity profiles of fourteen selected medicinal plants from Rural Venda communities in South Africa against fifteen clinical bacterial species. Afr J Biotech 4:1443–1451

Samie A, Tambani T, Harshfield E, Green E, Ramalivhana JN, Bessong PO (2010) Antifungal activities of selected venda medicinal plants against *Candida albicans*, *Candida krusei* and *Cryptococcus neoformans* isolated from South African. Afr J Biotech 9:2965–2976

Sauton M, Miyamoto T, Lacaille MA (2006) Bioactive steroidal saponins from *Smilax medica*. Planta Med 72:667–669

Saxena G, McCutcheon AR, Farmer S, Towers GHN, Hancock REW (1994) Antimicrobial constituents of *Rhus glabra*. J Ethnopharmacol 42:95–99

Saxena VK, Sharma RN (1999) Antimicrobial activity of the essential oil of *Toddalia Asiatica*. Fitoterapia 70:64–66

Schultes RE (1978) The kingdom of plants. In: Thomson WAR (ed) Medicines from the Earth. McGraw-Hill Book Co, New York, p 208

Shankaracharya NB, Rao LJM, Puranaik J, Nagalakshmi (2000) Studies on chemical and technological aspects of Indian dill seed (Anethumsowa. Rxb). J Food Sci M 37: 368–372

Shenoy SR, Kameshwari MN, Swaminathan S, Gupta MN (2006) Major antifungal activity from the bulbs of Indian squill *Urginea indica* is a chitinase. Biotechnol Prog 22:631–637

Shin S (2004) Essential oil compounds from *Agastache rugosa* as antifungal agents against *Trichophyton* species. Arch Pharm Res 27:295–299

Shin S, Kang CA (2003) Antifungal activity of the essential oil of *Agastache rugosa* Kuntze and its synergism with ketoconazole. Lett Appl Microbiol 36:111–115

Singh SP, Shukla HS, Singh RS and Tripathi SC (1986) Antifungal properties of essential oil of Ageratum conyzoides L. Nat Acas Sci Letters 9: 97–99

Singh NV, Azmi S, Maurya S, Singh UP, Jha RN, Pandey VB (2003) Two plant alkaloids isolated from *Corydalis longipes* as potential antifungal agents. Folia Microbiol 48:605–609

Singh UP, Sharma BK, Mishra PK, Ray AB (2000) Antifungal activity of Venenatine, an indole alkaloid isolated from *Alstonia venenata*. Folia Microbiol 45:173–176

Stern JL, Hagerman AE, Steinberg PD, Mason PK (1996) Phlorotannin-protein interactions. J Chem Ecol 22:1887–1899

Srinivas PV, Rao RR, Rao JM (2006) Two new tetracyclic triterpenes from the heartwood of Ailanthus excelsa Roxb. Chem Biodivers 3:930–934

Sunthitikawinsakul A, Kongkathip N, Kongkathip B, Phonnakhu S, Daly JW, Spande TF, Nimit Y, Rochanaruangrai S (2003) Coumarins and carbazoles from *Clausena excavata* exhibited antimycobacterial and antifungal activities. Planta Med 69:155–157

Taira T, Toma N, Ishihara M (2005) Purification, characterization, and antifungal activity of chitinases from pineapple (*Ananas comosus*) leaf. Biosci Biotechnol Biochem 69:189–196

Thornes RD, O'Kennedy R, Thornes RD (1997) Coumarins: biology, applications and mode of action. Wiley, New York p 256

Thouvenel C, Gantier JC, Duret P, Fourneau C, Hocquemiller R, Ferreira ME, Rojas de Arias A, Fournet A (2003) Antifungal compounds from *Zanthoxylum chiloperone* var. *angustifolium*. Phytother Res 17:678–680

Tostao Z, Noronha JP, Cabrita EJ, Medeiros J, Justino J, Bermejo J, Rauter AP (2005) A novel pentacyclic triterpene from *Leontodon filii*. Fitoterapia 76:173–80

Treadway S (1998) Exploring the Universe of Ayurvedic botanicals to manage bacterial infections. Clin Nutr Insights 6:1–3

Tripathi RD, Srivastava HS, Dixit SN (1978) A fungitoxic principle from the leaves of *Lawsonia inermis* Lam. Cell Mol Life Sci 34:51–52

Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M, Tanaka T, Iinuma M (1996) Comparative study on the antibacterial activity of phytochemical flavanones against methicillin resistant *Staphylococcus aureus*. J Ethnopharmacol 50:27–34

Wang H, Ng TB (2001) Novel antifungal peptides from *Ceylon spinach* seeds. Biochem Biophys Res Commun 288:765–770

Wang H, Ng TB (2005) An antifungal protein from ginger rhizomes. Biochem Biophys Res Commun 336:100–4

Yadav V, Mandhan R, Pasha Q, Pasha S, Katyal A, Chhillar AK, Gupta J, Dabur R, Sharma GL (2007) An antifungal protein from *Escherichia coli*. J Med Microbiol 56: 637–44

Ye XY, Ng TB (2002) Purification of angularin, a novel antifungal peptide from *Adzuki* beans. J Pept Sci 8:101–106

Ye XY, Ng TB, Rao PF (2002) Cicerin and arietin, novel chickpea peptides with different antifungal potencies. Peptides 23:817–822

Ye XY, Ng TB, Tsang PW (2001) Isolation of a homodimeric lectin with antifungal and antiviral activities from red kidney bean (*Phaseolus vulgaris*) seeds. J Protein Chem 20:367–375

Yemele M, Krohn K, Hussain H, Dongo E, Schulz B, Hu Q (2006) Tithoniamarin and tithoniamide: a structurally unique isocoumarin dimer and a new ceramide from *Tithonia diversifolia*. Nat Prod Res 20:842–849

Yenesew A, Derese S, Midiwo JO, Bii C, Heydenreich M, Peter MG (2005) Antimicrobial flavonoids from the stem bark of *Erythrina burttii*. Fitoterapia 76:469–472

Zhang JD, Cao YB, Xu Z, Sun HH, An MM, Yan L, Chen HS, Gao PH, Wang Y, Jia XM, Jiang YY (2005) *In vitro* and in vivo antifungal activities of the eight steroid saponins from *Tribulus terrestris* Linn. with potent activity against fluconazole-resistant fungal pathogens. Biol Pharm Bull 28:2211–2215

Zhang JD, Xu Z, Cao YB, Chen HS, Yan L, An MM, Gao PH, Wang Y, Jia XM, Jiang YY (2006) Antibacterial and antifungal activities of different parts of *Tribulus terrestris* Linn. growing in Iraq. J Ethnopharmacol 103:76–79

Chapter 12
Plants Used in Folk Medicine of Bangladesh for Treatment of Tinea Infections

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Abstract Tinea (often known as ringworm) is a fungal infection caused by various fungi like *Trichophyton rubrum*, *T. tonsurans*, *T. interdigitale*, *Microsporum canis*, and *Epidermophyton floccosum*. These fungi are known as dermatophyte fungi. Tinea can affect any age group but usually is seen in children. Patients suffering from tinea versicolor are quite common in the occasional rural clinics or even city hospitals of Bangladesh. The rural people of Bangladesh depend on folk medicinal practitioners, otherwise known as Kavirajes for treatment of various ailments, including tinea infections. The main characteristic of Kavirajes is that they depend on administration (topical or oral) of various medicinal plants for treatment of tinea infections. Ethnomedicinal surveys were carried out among the Kavirajes of Bangladesh, as well as tribal medicinal practitioners of more than 20 indigenous communities of the country to document the use of medicinal plants used for treatment of tinea infections by these healers. A total of 26 plants distributed into 23 families were found in the various surveys to be used by the Kavirajes. Although various plant parts were used, leaves constituted the majority of uses. The present review focuses on the medicinal plants used by Kavirajes and tribal medicinal practitioners for treatment of tinea diseases in Bangladesh along with any relevant scientific findings on the anti-fungal activities of the plants, which can validate the plants' traditional uses. The need for novel, efficacious, safer, and broader spectrum antifungal agents cannot be over emphasized. From this perspective, the above-mentioned medicinal plants may lend themselves to systematic modern scientific explorations in pursuit of novel antifungal agents.

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12.1 Introduction

Tinea (often known as ringworm) is a fungal infection caused by various fungi like *T. rubrum*, *T. tonsurans*, *T. interdigitale*, *M. canis*, and *E. floccosum*. These fungi are known as dermatophyte fungi. Tinea is referred to by various names depending on the part of the body infected with the fungi. Tinea corporis is fungal infection of the skin of the body, tinea capitis refers to scalp, tinea cruris (jock itch) refers to groin, tinea pedis (athlete's foot) refers to feet, and tinea unguium (onychomycosis) refers to fungal infections of the nails. Tinea can affect any age group but usually is seen in children. The fungi may spread from person to person (anthropophilic), from animal to person (zoophilic), or from the soil to person (geophilic). Heat and moisture help the fungi to grow and survive. Thus, tinea infections often occur in the groin or between toes, where excessive sweat or moisture can lead to a damp surface on the skin, enabling the fungi to infect and proliferate. Tinea infections may cause red skin rash that forms a ring, which can be itchy or cause cracking of the skin.

Bangladesh is a hot and humid country with nearly 9 months of the year having high temperatures with humidity in excess of 80 % or with monsoon rains. The majority of the population is rural based with agriculture being their chief occupation. Agriculture necessitates working in fields under the hot sun or during rains, conditions leading to excessive sweating or wetting of the entire body. Moreover, the village people live in earthen houses with thatched or tin roofs; since the roofs are not changed periodically due to the poor economic status of the people, they leak and allow rain to enter the house, leading to creation of damp conditions within the rooms. Under such circumstances, skin diseases are common. Bangladesh also has a number of tribes living in the forested hilly regions of the southeast, north central, and northeast regions of the country. Tribal people also develop skin infections like tinea. Patients suffering from tinea versicolor are quite common in the occasional rural clinics or even city hospitals (Sadeque et al. 1995; Muhammad et al. 2009). Tinea versicolor (also known as dermatomycosis furfuracea, pityriasis versicolor, and tinea flava) is a condition characterized by a rash on the trunk and proximal extremities. Recent research has shown that the majority of tinea versicolor is caused by the *Malassezia globosa* fungus, although *M. furfur* is responsible for a small number of cases.

Since allopathic doctors or modern clinics and hospitals are mostly absent in the villages, the rural people depend on folk medicinal practitioners, otherwise known as Kavirajes for treatment of various ailments, including tinea infections. The main characteristic of Kavirajes is that they depend on administration (topical or oral) of various medicinal plants for treatment of a diverse variety of ailments. Folk medicine is considered to have been present within the country for centuries. The medicinal plant knowledge accumulated by any particular Kaviraj is passed on to successive generations (usually within the family), and thus over the centuries, Kavirajes have gained considerable knowledge on the medicinal plants found in the vicinity of their habitat. Our various ethnomedicinal surveys conducted among

the Kavirajes of different regions and various tribes of Bangladesh have indicated that skin disorders are quite common among the rural population and tribes and that these disorders are treated by the Kavirajes and tribal medicinal practitioners with medicinal plants (Rahmatullah et al. 2010a, b, c, d, e, f, g; Rahmatullah and Biswas 2012; Rahmatullah et al. 2012a, b, c). The present review shall focus on the medicinal plants used by Kavirajes and tribal medicinal practitioners for treatment of tinea diseases in Bangladesh along with any relevant scientific findings on the anti-fungal activities of the plants, which can validate the plants traditional uses.

12.2 Ethnomedicinal Surveys and Identification of Plants

The ethnomedicinal surveys were carried out in randomly selected villages covering almost all the 64 districts of Bangladesh and over 20 tribes or indigenous communities present within the country. The Kavirajes were asked to describe the skin disorders that they treated, and tinea infections among the patients were visually identified. Kavirajes were next specifically requested to name and point out the plants that they used for treatment of tinea infections through guided field walks through areas from where they collected their plants. Plant specimens were photographed and collected on the spot and identified at the Bangladesh National Herbarium at Dhaka.

12.3 Medicinal Plants Used for Treatment of Tinea Infections

A total of 26 plants distributed into 23 families were found in the various surveys to be used by the Kavirajes. Although various plant parts were used, leaves constituted the majority of uses. The results are shown in Table 12.1. The mode of use was fairly simple. Whole plants or plant parts (singly or in combination) were macerated to obtain juice followed by topical application of the juice to the infected area. There was no generalized time period for treatment; juice was advised to be applied at least once a day following cleansing of the infected area with water, and applications were continued till cure. There was only one exception to this rule. The fruits of *Terminalia belerica* were advised to be taken orally; at the same time, juice obtained from macerated leaves and barks of the plant were topically applied to infected areas. The following sections shall discuss the anti-fungal properties of the various plants used by the Kavirajes and tribal medicinal practitioners for treatment of tinea infections.

Table 12.1 Medicinal plants used for treatment of tinea infections by folk medicinal practitioners of Bangladesh

Botanical name	Family	Local name	Parts used
Justicia gendarussa L. Synonyms: *Gendarussa vulgaris* Bojer, *Gendarussa vulgaris* Nees English: Gendarussa	Acanthaceae	Bish zara	Whole plant
Aloe vera (L.) Burm.f. Synonyms: *Aloe barbadensis* Mill., *Aloe vulgaris* Lam., *Aloe indica* Royle, *Aloe lanzae* Tod. English: Indian Aloe, Barbados Aloe	Aloaceae	Ghritokumari	Whole plant
Tabernaemontana divaricata (L.) R. Br. ex Roemer and J.A. Schultes Synonyms: *Ervatamia coronaria* (L.) Stapf, *Tabernaemontana coronaria* (L.) Willd. English: Pinwheel flower, Crepe jasmine	Apocynaceae	Roma phool	Seed
Cocos nucifera L. Synonyms: *Palma cocos* Miller English: Bahia coconut palm (Brazil), coconut, coconut palm, copra (product)	Arecaceae	Narikel	Young leaf
Impatiens balsamina L. Synonyms: *Balsamina hortensis* Desp. English: Rose balsam, garden balsam, impatiens, spotted snapweed, touch-me-not	Balsaminaceae	Dopati	Leaf
Heliotropium indicum L. Synonyms: *Tiaridium indicum* (L.) Lehm English: Indian heliotrope	Boraginaceae	Hatishur	Leaf
Carica papaya L. Synonyms: *Carica hermaphrodita* Blanco, *Carica mamaja* Vellero, *Carica vulgaris* DC., *Papaya carica* Gaertner, *Papaya papaya* Karsten, *Papaya sativa* Tussac, *Papaya vulgaris* A. DC. English: Pawpaw, Papaya	Caricaceae	Papay	Whole plant, leaf, gum
Chenopodium ambrosioides L. Synonyms: *Ambrina ambrosioides* (L.) Spach, *Atriplex ambrosioides* Crantz, *Blitum ambrosioides* (L.) Beck, *Chenopodium suffruticosum* Willd., *Dysphania ambrosioides* (L.) Mosyakin and Clemants, *Teloxys ambrosioides* (L.) W. A. Weber English: American wormseed, Bluebush, Indian goosefoot, Jerusalem tea, Jesuit's tea, Mexican tea, Spanish tea, Wormseed	Chenopodiaceae	Gobra bhang	Leaf, seed

(continued)

Table 12.1 (continued)

Botanical name	Family	Local name	Parts used
Mesua ferrea L.	Clusiaceae	Nageshwar	Leaf
Synonyms: *Calophyllum nagassarium* Burm. F., *Mesua coromandelina* Wight, *Mesua nagassarium* (Burm. F.) Kosterm., *Mesua pedunculata* Wight, *Mesua roxburghii* Wight, *Mesua sclerophylla* Thw., *Mesua speciosa* Choisy			
English: Ceylon Ironwood, Indian rose chestnut			
Terminalia belerica (Gaertn.) Roxb.	Combretaceae	Bohera	Leaf, bark, fruit
Synonyms: *Myrobalanus bellirica* Gaertn., *Myrobalanus laurinioides* Kuntze, *Terminalia angustifolia* Blanco, non Jacq., *Terminalia attenuata* Edgew., *Terminalia edulis* Blanco, *Terminalia moluccana* Roxb., *Terminalia punctata* Roth			
English: Bastard myrobalan, Behere, Belleric myrobalan			
Ipomoea fistulosa Mart. ex Choisy	Convolvulaceae	Dhol kolmi	Leaf, bark, gum
Synonyms: *Ipomoea carnea* Jacq. subsp. *fistulosa* (Mart. ex Choisy) D. F. Austin			
English: Bush morning glory			
Kalanchoe pinnata (Lam.) Pers.	Crassulaceae	Pathorkuchi	Whole plant
Synonyms: *Bryophyllum pinnatum* (Lam.) Oken, *Cotyledon pinnata* Lam., *Bryophyllum calycinum* Salisb., *Bryophyllum germinans* Blanco, *Cotyledon calycina* Salisb. Roth, *Cotyledon calyculata* Solander ex De Candolle, *Cotyledon rhizophilla* Roxb., *Crassuvia floripendia* Commers. Ex Hiern, *Crassula pinnata* L.f., *Sedum madagascariense* (H.Perrier) H.Obla, *Verea pinnata* (Lam.) Spreng.			
English:			
Juniperus chinensis L.	Cupressaceae	Juniperus	Whole plant
Synonyms: *Juniperus chinensis* L. var. *columnaris* D. Fairchild			
English: Chinese juniper, Chinese cedarwood			
Phyllanthus niruri L.	Euphorbiaceae	Bhui amla	Whole plant
Synonyms: *Diasperus niruri* (L.) Kuntze, *Phyllanthus fraternus* Webster, *Phyllanthus filiformis* Pavon ex Baillon			
English: Small gooseberry			
Cassia alata L.	Fabaceae	Dard-mordon	Leaf
Synonyms: *Senna alata* (L.) Roxb., *Herpetica alata* (L.) Raf., *Cassia bracteata* L.f., *Cassia herpetica* Jacq.			
English: Guajava, Candlebush, Candlestick senna, Christmas candle, Ringworm bush, Ringworm senna, Ringworm shrub, Seven-golden-candlesticks			

(continued)

Table 12.1 (continued)

Botanical name	Family	Local name	Parts used
Cassia tora L. Synonyms: *Senna tora* (L.) Roxb., *Cassia obtusifolia* L. English: Ring worm plant, Foetid cassia, Sickle senna, Wild senna	Fabaceae	Charkada	Seed, leaf
Erythrina variegata L. Synonyms: *Erythrina indica* Lam., Erythrina orientalis (L.) Merr English: Tiger's claw	Fabaceae	Maandial gach	Leaf, bark
Lablab purpureus (L.) sweet Synonyms: *Dolichos lablab* L., *Dolichos benghalensis* Jacq., *Dolichos purpureus* L., *Lablab niger* Medikus, *Lablab purpurea* (L.) Sweet, *Lablab vulgaris* (L.) Savi, *Vigna aristata* Piper English: Banner bean, Black bean, Bonavist bean	Fabaceae	Shim	Leaf
Gynocardia odorata R.Br. Synonyms: *Chaulmoogra odorata* (R.Br.) Roxb English: Chaulmoogra, false gynocardia oil	Flacourtiaceae	Chal moghra	Leaf, bark, seed
Asparagus racemosus Willd. Synonyms: *Asparagus rigidulus* Nakai, *Asparagus schoberioides* Kunth, *Asparagus volubilis* Buch.-Ham., *Protasparagus racemosus* (Willd.) Oberm English: Indian asparagus, Sataver white, Sataver yellow, Wild asparagus	Liliaceae	Shotomuli	Bark
Lawsonia inermis L. Synonyms: *Lawsonia alba* Lam. English: Henna	Lythraceae	Mehedi	Leaf, stem, bark
Artocarpus heterophyllus Lam. Synonyms: *Artocarpus integer* auct., *Artocarpus integrifolia* auct., *Artocarpus jaca* Lam. English: Jackfruit, Jackfruit tree	Moraceae	Kanthal	Leaf
Piper betle L. syn. *Chavica betle* Miq., *Chavica auriculata* Miq. English: Betel, Betel pepper, Betelvine, Betel vine	Piperaceae	Paan	Leaf
Murraya koenigii (L.) Spreng syn. *Bergera koenigii* L., *Chalcas koenigii* (L.) Kurz English: Curry leaf tree	Rutaceae	Keri pata	Leaf

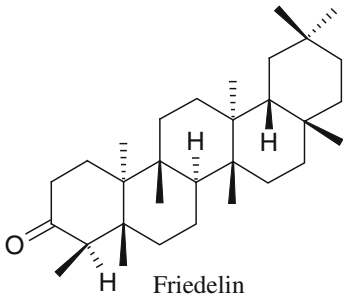
(continued)

Table 12.1 (continued)

Botanical name	Family	Local name	Parts used
Nyctanthes arbor-tristis L., syn. *Bruscha macrocarpa* Bertol., *Nyctanthes arbor-tristis* var. *Dentata* Hort. ex Moldenke, *Nyctanthes dentata* Blume, *Nyctanthes tristis* Salisb., *Parilum arbor-tristis* Gaertn., *Scabrita triflora* L. English: Night jasmine	Verbenaceae	Shefali	Leaf
Curcuma aromatica Salisb. English: Curcuma, Aromatic turmeric, Yellow zedoary, Wild turmeric	Zingiberaceae	Aam ada	Tuber

12.3.1 Justicia Gendarussa

The leaves are reported to contain friedelin (Shikha et al. 2010). Friedelin isolated from *Azima tetracantha* Lam. (Salvadoraceae), reportedly showed high antifungal activity against *T. rubrum* and *Curvularia lunata* with MIC value of 62.5 μg/ml (Duraipandiyan et al. 2010). The compound isolated from *Syzygium jambos* (L.) Alston (Myrtaceae) demonstrated anti-dermatophytic activity against three dermatophyte species, namely, *M. audouinii*, *T. mentagrophytes*, and *T. soudanense* (Kuiate et al. 2007).



Friedelin

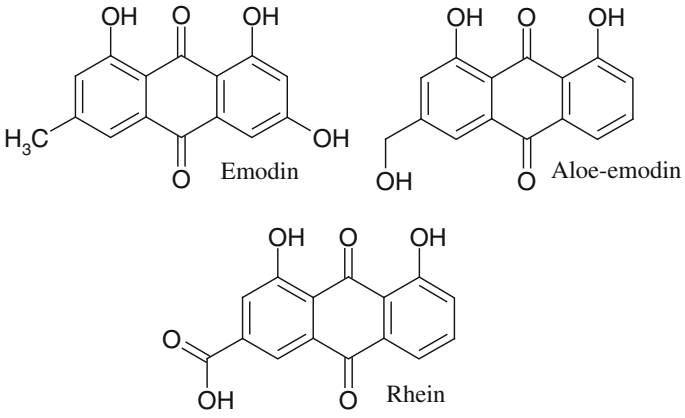
12.3.2 Aloe Vera

Various *Aloe* species are used in traditional medicinal system of Nigeria for various skin diseases including tinea capitis (Ajose 2007). Extract of fresh leaves of the plant showed anti-fungal properties by inhibiting the growth and germ tube formation by *Candida albicans* (Bernardes et al. 2012). A 14 kDa protein has been isolated from leaf gel possessing antifungal properties against *C. paraprilosis*, *C. krusei*, and *C. albicans* (Das et al. 2011). Hydroalcoholic extract of fresh leaves reportedly inhibited the mycelia growth of *Botrytis gladiolorum*, *Fusarium oxysporum* f.sp. *gladioli*, *Heterosporium pruneti*, and *Penicillium gladioli* (Rosca-Casian et al. 2007). Extracts of both fresh and dry leaves of *Aloe eru* A. Berger, *A. vera* L. Webb and Berth, and *A. arborescens* Mill reportedly exhibited anti-fungal activity against *Cladosporium herbarum* and *F. moniliforme* (Ali et al. 1999).

The antifungal activity of *A. vera* leaves is possibly due to the presence of anthraquinone alkaloids like emodin, physcion, rhein, and aloe-emodin. Rhein, physcion, and aloe-emodin isolated from *Rheum emodi* Wall. ex Meisn. (Polygonaceae) rhizomes have been shown to exhibit anti-fungal activity against *C. albicans*, *Cryptococcus neoformans*, *T. mentagrophytes*, and *Aspergillus fumigatus* with MICs of 25–250 microg/ml (Agarwal et al. 2000). Various anthraquinones (emodin, physcion, aloe emodin and rhein) isolated from *Cassia tora* L. (Fabaceae) showed anti-fungal activities against the phytopathogenic fungi *B. cineria*, *Erysiphe*

graminis, *Phytophthora infestans*, *Puccinia recondita*, *Pyricularia grisea*, and *Rhizoctonia solani* (Kim et al. 2004). Antifungal activity has also been reported for emodin isolated from *Rhamnus frangula* L. (Rhamnaceae) (Manojlovic et al. 2005).

Overall, anthraquinones are noted for their anti-fungal properties. To cite a few examples, purpurin (1,2,4-trihydroxy-9,10-anthraquinone) isolated from *Rubia tinctorum* L. (Rubiaceae) has been shown to be active against six *Candida* species (Kang et al. 2010). A new anthraquinone, 1-methyl-2-(3′-methyl-but-2′-enyloxy)-anthraquinone, isolated from seeds of *Aegle marmelos* Correa (Rutaceae) exhibited significant anti-fungal activity against pathogenic strains of *Aspergillus* species and *C. albicans* (Mishra et al. 2010). Anthraquinones isolated from the roots of *Prismatomeris malayana* (Roxb.) K. Schumann (Rubiaceae) also reportedly demonstrated anti-fungal activities (Tuntiwachwuttikul et al. 2008).

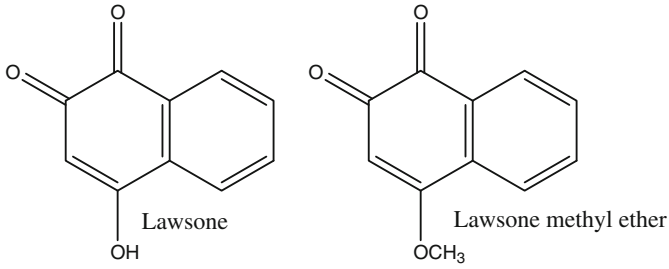


12.3.3 *Cocos nucifera*

Oil obtained from fruit pulp of *Cocos nucifera* (coconut oil) has been found to be effective against experimentally produced dermatophytic infection on hairy skin in guinea pigs (Abraham et al. 1975). Coconut oil has effectiveness against *Tinea capitis* (tinea infections on the scalp). A 10 kDa anti-fungal peptide has been isolated from coconut flesh, which showed inhibitory actions against *F. oxysporum*, *Mycosphaerella arachidicola*, and *Physalospora piricola* (Wang and Ng 2005). The alcoholic extract of coconut shell also has been reported to possess anti-fungal properties (Venkataraman et al. 1980). Coconut kernel and tender coconut water have been reported to numerous properties including anti-bacterial, anti-fungal, anti-parasitic, and anti-viral (DebMandal and Mandal 2011). Coconut oil contains oleic acid, which can be the basis of its anti-fungal activity against dermatophytic infections on hairy skin including the scalp (Abraham et al. 1975).

12.3.4 *Impatiens balsamina*

Naphthoquinones like lawsone and lawsone methyl ether are two of the main naphthoquinones in leaf extracts of *Impatiens balsamina* (Sakunphueak and Panichayupakaranant 2012). Anti-microbial activities of these two compounds against dermatophytic fungi, yeast, aerobic bacteria, and facultative anaerobic and anaerobic bacteria have been demonstrated with the second compound showing higher activity against dermatophytic fungi and *C. albicans*. Ethanol extracts of flower petals reportedly showed anti-pruritic and anti-dermatitic effects, which have been attributed to the presence of kaempferol 3-rutinoside and lawsone (2-hydroxy-1,4-naphthoquinone) in the extract (Oku and Ishiguro 2001). Lawsone, isolated from *Lawsonia inermis* L. (Lythraceae), has been reported to exhibit fungicidal activity with a wide fungitoxic spectrum and nonphytotoxicity (Tripathi et al. 1978). The anti-fungal activity of lawsone methyl ether has been reported against *Candida* species (Sritrairat et al. 2011). A set of related anti-microbial peptides (Ib-AMPs) have been reported in seeds, which demonstrated activity against various yeast and fungal strains (Thevissen et al. 2005).



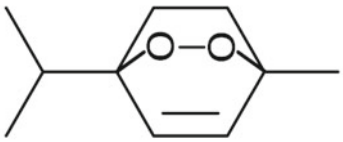
12.3.5 *Carica papaya*

A chitinase with a molecular weight of 26.2 kDa has been isolated from fruits, which showed anti-fungal activity by completely inhibiting the spore germination of *Alternaria brassicicola* (Chen et al. 2007). A mixture of *Carica papaya* latex sap and fluconazole showed a synergistic action on the inhibition of *C. albicans* growth (Giordani et al. 1997). The fungicidal activity against *C. albicans* has been shown for plant latex sap alone (Giordani et al. 1996). The anti-fungal activity of latex sap could be due to the presence of fungal cell wall hydrolyzing latex proteins like α-D-mannodidase and N-acetyl-β-D-glucosoaminidase (Giordani et al. 1991).

12.3.6 *Chenopodium ambrosioides*

The essential oil obtained from the plant showed strong fungitoxicity against two dermatophytic fungi, *T. rubrum* and *M. gypseum*. Experimental ringworm in guinea pigs could be cured within 7–12 days in ointment formulation (Kishore et al. 1993). Anti-fungal activity of methanol and n-hexane extracts of three *Chenopodium* species, namely, *Chenopodium album* L., *C. ambrosioides* L., and *C. murale* L. has been reported against a soil-borne fungal plant pathogen, *Macrophomina phaseolina* (Javaid and Amin 2009). A synergistic activity has been reported with essential oils of *Cymbopogon martini* and *C. ambrosioides* against induced ringworm infection in guinea pig model in vivo and dermatophytes and some filamentous fungi in vitro (Prasad et al. 2010). Volatile components isolated from leaves inhibited hyphal growth and sclerotia formation of *Sclerotium rolfsii* (Singh 2005).

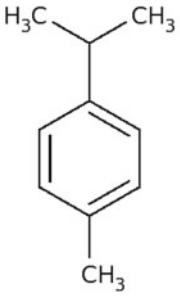
The essential oil from the plant has been evaluated and found to be fungitoxic against *A. flavus*, *A. glaucus*, *A. niger*, *A. ochraceus*, *Colletotrichum gloesporioides*, *C. musae*, *F. oxysporum*, and *F. semitectum*. The identified components of the oil included p-cymene, carvacrol, (Z)-ascaridole, and (E)-ascaridole. It has been suggested that ascaridoles were the principal fungitoxic components of the oil (Jardim et al. 2008). Essential oil obtained from leaves exhibited broad spectrum fungitoxic activity against *A. flavus*, *A. niger*, *A. fumigatus*, *Botryodiplodia theobromae*, *F. oxysporum*, *S. rolfsii*, *M. phaseolina*, *C. cladosporioides*, *Helminthosporium oryzae*, and *Pythium debaryanum* at 100 μg/ml (Kumar et al. 2007).



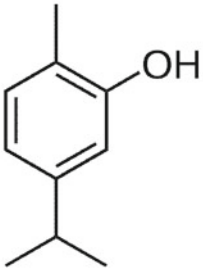
Ascaridole

Besides ascaridole, p-cymene and carvacrol may also contribute to the observed anti-fungal activity. These two compounds are present in essential oil of *C. ambrosioides* as described above. The essential oil of fruits of *Cuminum cyminum* L. (Apiaceae) tested positive for inhibition of dermatophytes (*T. rubrum* was the most inhibited dermatophyte) and other fungi including yeasts and some *Aspergillus* species; p-cymene was one of the most abundant component of the oil and may have contributed to its anti-fungal properties (Romagnoli et al. 2010). Carvacrol and p-cymene were also major components of essential oil from *Thymus* x *vicisoi* (Pau) R. Morales (Lamiaceae), which demonstrated anti-fungal activity against *C. albicans*, *Cryptococcus*, *Aspergillus*, and dermatophyte species. Fungicidal activity was caused through disruption of cytoplasmic membrane integrity leading to leakage of vital intracellular components (Vale-Silva et al. 2010). Very strong anti-fungal activities were obtained with essential oil from *T. vulgaris*

L. and *T. tosevii* L., p-cymene and carvacrol, respectively, being the major components of oil from the two species of plants (Soković et al. 2009). Carvacrol and p-cymene, components of essential oils obtained from *T. vulgaris*, *T. zygis* subspecies *zygis* and *T.mastichina* subspecies *mastichina*, reportedly showed anti-fungal activity against *Candida* species (Pina-Vaz et al. 2004). The essential oil from aerial parts of *Rosmarinus officinalis* L. (Lamiaceae) collected from Turkey and which exhibited anti-fungal activity against *A. alternata*, *B. cinerea*, and *F. oxysporum* also had p-cymene as the major component (Ozcan and Chalchat 2008). Carvacrol and p-cymene were among the components of essential oil obtained by hydrodistillation of aerial parts of Turkish *Origanum acutidens* (Hand-Mazz) Ietsw. (Lamiaceae), and which displayed anti-fungal and insecticidal properties (Kordali et al. 2008). Strong anti-fungal activity against *F. oxysporum* was displayed by p-cymene isolated from *Bunium persicum* (Boiss.) B. Fedtsch. (Apiaceae) (Sekine et al. 2007). Taken together, the results strongly suggest that *C. ambrosioides* and other species belonging to the *Chenopodium* genera may be good candidates for isolation of compounds like ascaridole, carvacrol and p-cymene, which compounds may prove to be of therapeutic value for treatment of dermatophytic fungi including tinea infections.



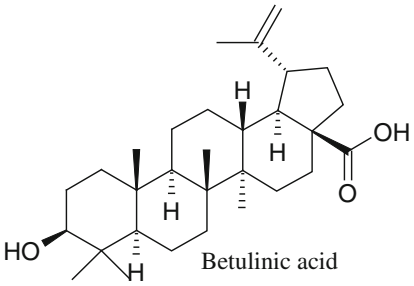
p-Cymene



Carvacrol

12.3.7 Mesua ferrea

The root barks of the plant contain friedelin and betulinic acid (Teh et al. 2011). The anti-fungal activity of friedelin has been discussed in Sect. 12.3.1. Betulin has been shown to have significant fungistatic activity against some filamentous fungi (Jasicka-Misiak et al. 2010). Betulin and betulinic acid, isolated from *Eugenia umbelliflora* Berg. (Myrtaceae) exhibited selective antifungal activity against dermatophytes like *E. floccosum*, *M. canis*, *M. gypseum*, *T. rubrum*, and *T. mentagrophytes* (Machado et al. 2009). Anti-fungal activity has also been reported for betulinic acid, isolated from the South African plant, *Curtisia dentata* (Burm.f.) C.A. Sm. (Curtisiaceae) (Shai et al. 2008).

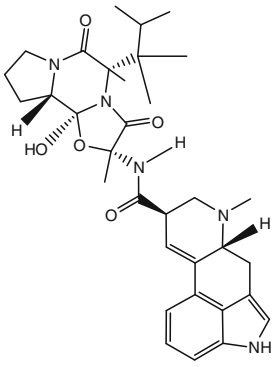


Betulinic acid

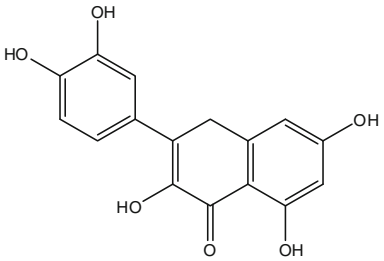
Xanthones appear to be another group of compounds present in *Mesua ferrea* which can account for the use of this plant by the Kavirajes of Bangladesh for treatment of tinea infections. Mesuaxanthone-A, mesuaxanthone-B, and euxanthone have been isolated from the plant (Gopalakrishnan et al. 1980). Various xanthones isolated from plants like *Hypericum perforatum* L. subsp. *angustifolium* (DC.) Gaudin (Clusiaceae) and *Rhus coriaria* L. (Anacardiaceae) have been shown to possess anti-fungal activity (Tocci et al. 2011; Singh et al. 2011). Another xanthone, α-mangostin has been found to be active against *C. albicans* with a minimum inhibitory concentration (MIC) of 1,000 μg per ml (Kaomongkolgit et al. 2009). Four antifungal active xanthones (1,6-dihydroxy-5-methoxyxanthone, 1-hydroxy-5,6-dimethoxyxanthone, 1-hydroxy-5-methoxyxanthone, and 1-methoxy-5-hydroxyxanthone) have been isolated from *Calophyllum thwaitesii* Planch. & Triana (Callophyllaceae) (Dharmaratne et al. 2009). Bioassay guided fractionation lead to isolation of two xanthones, cudraxanthone S and toxyloxanthone, from the roots of *Cudrania cochinchinensis* (Lour.) Kudô & Masam. (Moraceae). The xanthones showed inhibitory activities against *C. neoformans*, *A. fumigatus*, *C. glabrata*, and *A. nidulans* (Fukai et al. 2003). Antifungal xanthones, namely caledonixanthones E and F have been isolated from the stem bark of *C. caledonicum* Vieill. ex Planch. and Triana (Calophyllaceae). The xanthones were active against *A. fumigatus* and *C. albicans* (Morel et al. 2002).

12.3.8 Ipomoea fistulosa

An alkaloid, ergosine, has been reported from *Ipomoea fistulosa* leaves along with its anti-fungal activity (Shah and Mehta 2006). The flavonol glycoside, quercetin 3-glucoside has also been isolated from the leaves of the plant (Lamidi et al. 2000). There are evidences that quercetin and related glycosides may contribute to antifungal activity.



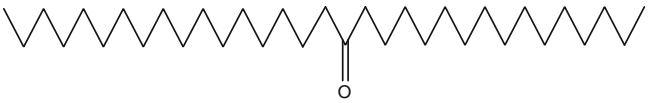
Ergosine



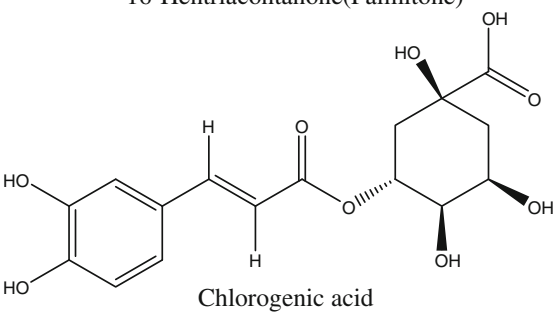
Quercetin

Quercetin-3-O-α-glucopyranosyl-(1 → 2)-β-D-glucopyranoside, isolated from *Mangifera indica* L. (Anacardiaceae) significantly suppressed fungal growth of *A. alternata*, *A. fumigatus*, *A. niger*, *M. phaseolina*, and *P. citrii* (Kanwal et al. 2010). Quercetin and chlorogenic acid were among the bio-active constituents isolated from *Adesmia aegiceras* Phil. (Fabaceae) showing inhibitory activity against *C. albicans* (Agnese et al. 2001).

A study of the nonpolar constituents of leaves of the plant revealed the presence of 16-hentriacontanone (Ahmad et al. 1998). 16-hentriacontanone (palmitone) has been isolated from the leaf cuticular wax of *Annona squamosa* L. (Annonaceae) and has been shown to possess anti-fungal activity (Shanker et al. 2007). Chlorogenic acid (3-caffeoylquinic acid) has been reported from the seeds of *I. fistulosa* (Sattar et al. 1995). Chlorogenic acid-based peptidomimetics has been shown to be a novel class of anti-fungals (Daneshtalab 2008). Derivatives of chlorogenic acid or its analogs exhibited significant potency against *C. neoformans*, *A. fumigatus*, and *Candida* species (Ma et al. 2007). The compound has been further used successfully for treatment of septic arthritis in mice model caused by *C. albicans* (Lee et al. 2008).



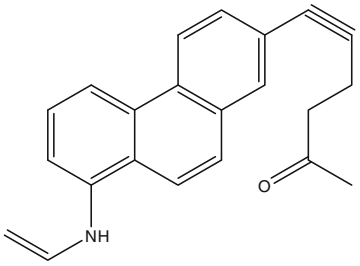
16-Hentriacontanone(Palmitone)



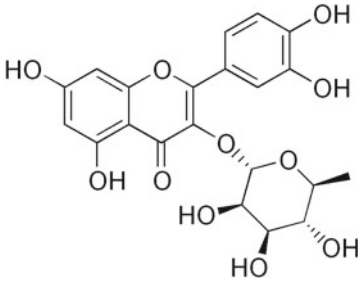
Chlorogenic acid

12.3.9 *Kalanchoe pinnata*

The flavonoids quercetin and quercetrin have been reported to be present in the plant (Muzitano et al. 2006; Cruz et al. 2008; Muzitano et al. 2011; Cruz et al. 2012). The anti-fungal activity of quercetin has been discussed in Sect. 12.3.8. Extract of *Rumex vesicarius* L. (Polygonaceae) and *Ziziphus spina-christi* (L.) Willd. (Rhamnaceae) containing quercetrin among other components, reportedly inhibited totally spore production and germination of *F. solani*, and to a lesser extent, that of *Drechslera biseptata* (Abu-Taleb et al. 2011). Quercitrin, isolated from *H. perforatum* L. (Hypericaceae), was observed to inhibit the growth of the phytopathogenic fungus, *H. sativum* (Lu et al. 2002). From the ethanolic extract of leaves, a novel phenenthrene alkaloid has been isolated and identified as 1-ethanamino 7 Hex-1-yne-51-one phenanthrene. The alkaloid has been shown to be active against *C. albicans* and *A. niger* (Okwu and Nnamdi 2011a).



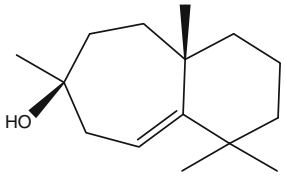
1-ethanamino7Hex-1-yne-5I-onephenanthrene



Quercitrin

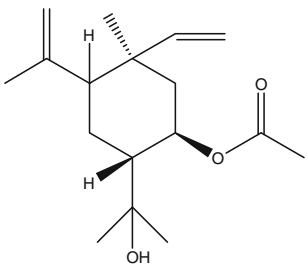
12.3.10 *Juniperus chinensis*

An anti-fungal compound, widdrol has been reported from the plant (Kwon et al. 2010a, b). Widdrol, isolated from *Juniperus lucayana* (Britton) R.P. Adams (Cupressaceae), was found to be the most active compound (among various sesquiterpenes and flavonoids) against *B. cinerea*, demonstrating a 71 % inhibition level on mycelia growth after 6 days (Nuñez et al. 2007). Anti-fungal activity of widdrol has also been reported against *B. cinerea* and *C. gloeosporioides*, both being plant pathogenic fungi (Nuñez et al. 2006).

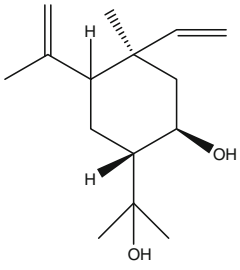


Widdrol

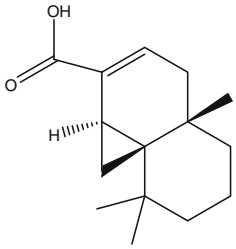
An anti-fungal screening done with 30 Japanese conifers against a wood blue-stain fungus (*Ceratocystis piceae*), a clothing fungus (*A. alternata*) and three dermatophytic fungi (*T. mentagrophytes*, *T. rubrum* and *E. floccosum*) revealed the presence of three active compounds in *J. chinensis pyramidalis*. The compounds were 8-acetoxyelemol, 8-hydroxyelemol and hinokiic acid and were all sesquiterpenes (Ohashi et al. 1994).



8-Acetoxyelemol



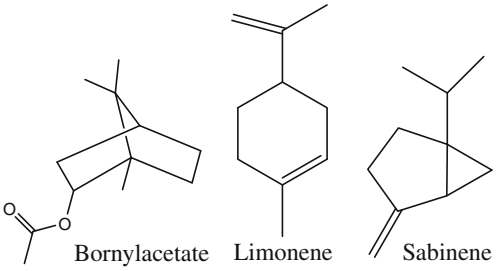
8-Hydroxyelemol



Hinokiicacid

Bornyl acetate, sabinene and limonene have been found in essential oil from this plant (Raina et al. 2005; Lee et al. 2009). There are evidences that these compounds may contribute to anti-fungal activities. The essential oils obtained from *Jasonia candicans* (Delile) Botsch (Asteraceae) and *J. montana* (Vahl.) Botsch showed marked anti-fungal activity against *T. mentagrophytes*, *C. neoformans*, and *C. albicans*. Borneol and bornyl acetate were among the major constituents of the essential oil obtained from the second plant (Hammerschmidt et al. 1993). Essential oil obtained from *Chamaecyparis obtusa* Siebold and Zucc. (Cupressaceae) containing bornyl acetate as the major component showed anti-fungal effect (Hong et al. 2004). In vivo anti-fungal activity against *Plasmopara halstedii* has been reported for the essential oil from *Bupleurum gibraltarium* Lam. (Umbelliferae); sabinene and α-pinene were the major components of the oil (Fernández-Ocaña et al. 2004). Essential oil obtained from *C. nootkatensis* (D. Don) Spach. (Cupressaceae) containing limonene as its major component showed promising activity against *C. albicans* (Palá-Paúl et al. 2009). The cyclic terpenes—limonene, menthol, menthone and thymol has been found to inhibit *F. verticillioides* MRC 826 growth and fumonisin B1 biosynthesis (Dambolena et al. 2008). Another anti-fungal

compound, quercetin, has been reported to be present in *J. chinensis* heartwood methanolic extract (Lim et al. 2002) (see Sect. 12.3.8 for discussion on quercetin as anti-fungal compound).



12.3.11 *Phyllanthus niruri*

Antifungal activity studies, with one exception, have not been carried out with this plant. Some activity with n-butanol, methanol, and aqueous extracts of the plant has been observed against the plant pathogenic fungi, *P. debaryanum* (Ambikapathy et al. 2011). However, other species belonging to the *Phyllanthus* genera have been studied for their anti-fungal activities. The essential oil from *Phyllanthus muellerianus* (Kuntze) Excell (Euphorbiaceae) has been shown to possess weak activity against *T. rubrum* LM 237 (Brusotti et al. 2012). Essential oil from *P. emblica* L. was effective against three pathogenic fungi. The major components of the oil were identified as β-caryophyllene, β-bourbonene, 1-octen-3-ol, thymol, and methyl-eugenol (Liu et al. 2009). Chloroform extract of the plant, *P. amarus* Schumach. and Thonn. showed significant inhibitory effect against the dermatophytic fungus *M. gypseum* (Agrawal et al. 2004). Anti-fungal activity has been observed with methylene chloride and methanol extracts of *P. acuminatus* Vahl (Goun et al. 2003). Dichloromethane extract of *P. piscatorum* Kunth showed inhibitory activity against *C. albicans* leading to the isolation of the arylnaphthalide lignin justicidin B, which inhibited the growth of the pathogenic fungi *A. fumigatus*, *A. flavus*, and *C. albicans* (Gertsch et al. 2003).

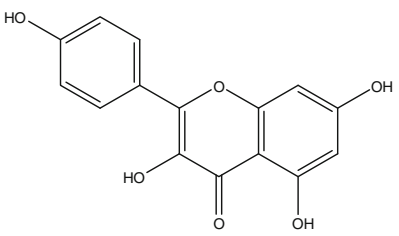
12.3.12 *Cassia alata*

The plant is considered of dermatologic importance in the traditional medicine of Nigeria (Ajose 2007). The plant is extensively used in folkloric medicines for treatment of ringworm infections and skin-related diseases in Tamil Nadu, India (Ponnusamy et al. 2010). A 10-year human study indicated that leaf extract of the plant can be reliably used as herbal medicine against *Pityriasis versicolor* (tinea versicolor) infections (Damodaran and Venkataraman 1994). Further purification

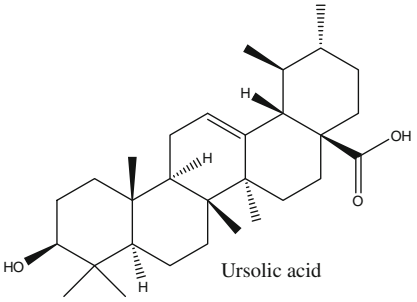
of methanol extract of leaves reportedly led to the isolation of the compounds, kaempferol, kaempferol 3-O-β-glucopyranoside, kaempferol 3-O-gentiobioside, and aloe emodin (Hazni et al. 2008). The anti-fungal activity of aloe emodin has been discussed earlier in Sect. 12.3.2. The chloroform extract of leaves also showed potent activity against *T. mentagrophytes*, while the ethyl acetate extract was active against *C. albicans* (Villaseñor et al. 2002). Ethanolic extract of leaves exhibited high activity against various species of dermatophytic fungi, but low activity against nondermatophytic fungi. An MIC of 125 mg/ml was noted against *T. mentagrophytes* var. *interdigitale*, *T. mentagrophytes* var. *mentagorophytes*, *T. rubrum*, and *M. gypseum*, while *M. canis* had MIC of 62.5 mg/ml (Ibrahim and Osman 1995). A combination of ethanol extracts of leaves of *C. alata* and *Ocimum sanctum* showed inhibitory activity against *Cryptococcus* species (Ranganathan and Balajee 2000).

Ethanol and water extracts of barks showed inhibitory activities against *C. albicans* (Somchit et al. 2003). Inhibitory activity of aqueous extract of the plant has been reported against *C. albicans* infections with MIC and minimum fungicidal concentration (MFC) of 0.39 and 60 mg/ml (Crockett et al. 1992).

Potential anti-fungal components of the plant include emodin, aloe emodin and rhein (see Sect. 12.3.2 for discussion of anti-fungal properties of these anthraquinones). Presence of these anthraquinones along with chrysophanol and physcion and a flavonoid—kaempferol has been confirmed in root extracts of the plant (Fernand et al. 2008; Villaroya and Bernal-Santos 1976). Kaempferol has been shown to inhibit in vitro various enzyme activities of *C. albicans*, which can lead to suppression of symptoms in cutaneous infections and accelerate elimination of the yeast from the site of inoculation (Yordanov et al. 2008).



Kaempferol

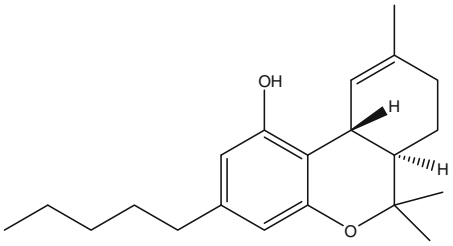


Ursolic acid

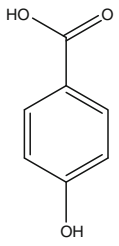
Investigation of compounds present in roots of the plant also showed the presence of the anthraquinones, viz., 1,5-dihydroxy-2-methylanthraquinone and alatinone in addition to n-octacosane, α-amyrin arachidate, tetracosanol, β-sitosterol, ursolic acid, and β-sitosterol-β-D-glucoside (Jain et al. 2010). The anti-fungal activity of *Melia azedarach* L. (Meliaceae) leaf methanolic extract against *Ascochyta rabiei*, the causative agent of destructive blight disease of chickpea has been investigated. Among the bio-active compounds present in the extract were ursolic acid and β-sitosterol (Jabeen et al. 2011). Ursolic acid as well as betulinic acid, isolated from *C. dentata* leaves also showed promising anti-fungal activity

against *C. albicans* (Shai et al. 2008). Ursolic acid, isolated from *Lythrum salicaria* L. (Lythraceae), was also reportedly active against the phytopathogenic fungus, *C. cucumerinum* (Becker et al. 2005). Ethanol extract of *Clytostoma ramentaceum* Bur. and K. Scum reportedly inhibited the growth of *A. niger* and *F. oxysporum* in standardized cultures, and which activity has been attributed to ursolic acid and 2-(3′,4′-dihydroxyphenyl) ethanol (Rocha et al. 2004).

4-Hydroxy benzoic acid has been reported from the leaves of *C. alata* (Rahman et al. 2006). The inhibitory activity of this compound has been demonstrated against *T. mentagrophytes* (MIC 0.5 mg/ml) and *E. floccosum* (MIC 0.5 mg/ml) (Duraipandiyan and Ignacimuthu 2007).



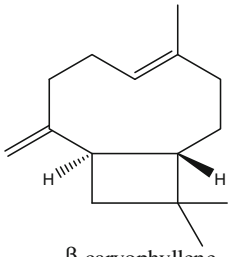
Dronabinol



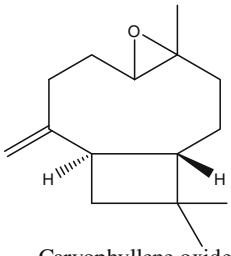
4-hydroxy Benzoic acid

Investigation of the bioactive constituents of the plant further led to the isolation of a cannabinoid alkaloid, 4-(butylamino)-10-methyl-6-hydroxy cannabinoid, dronabinol. The isolated compound inhibited *C. albicans* and *A. niger* (Okwu and Nnamdi 2011b).

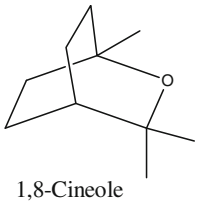
The major volatile constituents isolated from the essential oil obtained from leaves of the plant indicated presence of 1,8-cineole, *β*-caryophyllene, and caryophyllene oxide in addition to the minor constituents, limonene, germacrene D and α-selinene (Ogunwande et al. 2010). Various dermatophytes, *C. neoformans* and *C. albicans*, have been shown to the most sensitive against the essential oil obtained from *Lavandula viridis* L'Hér. (Lamiaceae) containing 1,8-cineole as the main constituent (Zuzarte et al. 2011). Essential oils obtained from *Eucalyptus largiflorens* F. Muell and *E. intertexta* R.T. Baker (Myrtaceae) having 1,8-cineole as the major component also demonstrated anti-microbial and anti-fungal properties (Safaei-Ghomi and Ahd 2010). Inhibitory activity of 1,8-cineole has further been demonstrated against yeast like and filamentous fungi (Pattnaik et al. 1997).



β-caryophyllene



Caryophyllene oxide

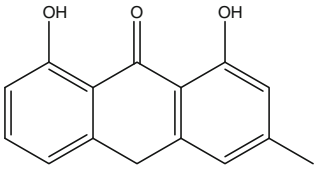


1,8-Cineole

The volatile extract of *Eryngium duriaei* subsp. *juresianum* (Apiaceae) demonstrated anti-fungal activity against several dermatophytic fungal species, namely *T. mentagrophytes*, *T. rubrum*, *E. floccosum*, *T. verrucosum*, *T. mentagrophytes* var. *interdigitale*, *M. canis*, and *M. gypseum*. The anti-fungal activities were attributed to caryophyllene-derived compounds like isocaryophyllen-14-al, 14-hydroxy-β-caryophyllene, caryophyllene oxide and E-β-caryophyllene (Cavaliero et al. 2011). Essential oil obtained from clove (*E. caryophyllata* Thunb. Myrtaceae) has been shown to possess inhibitory activity against human pathogenic yeasts (clinical *Candida* species); β-caryophyllene is one of the components of the oil (Chaieb et al. 2007). Volatile oil from rhizomes of *Zingiber nimmonii* (J. Graham) Dalzell has been reported to be a unique caryophyllene-rich oil, its major components being α-caryophyllene and β-caryophyllene. The oil reportedly showed significant inhibitory activity against various fungi like *C. albicans*, *C. glabrata*, and *A. niger* (Sabulal et al. 2006).

12.3.13 Cassia tora

Emodin has been reported to be present in seeds of *C. tora* (Jang et al. 2007). The anti-fungal properties of emodin have been discussed in Sect. 12.3.2. A major anti-fungal compound has been isolated from defatted seed powder and has been shown to be chrysophanic acid-9-anthrone. The compound was found to be active against *T. rubrum*, *T. mentagrophytes*, *M. canis*, *M. gypseum*, and *Geotrichum candidum* (Acharya and Chatterjee 1975).

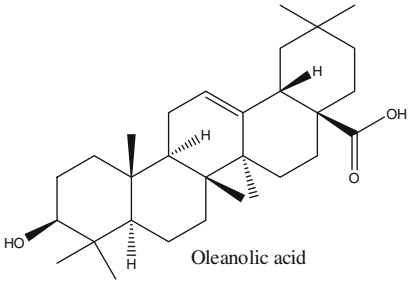


Chrysophanic acid-9-anthrone

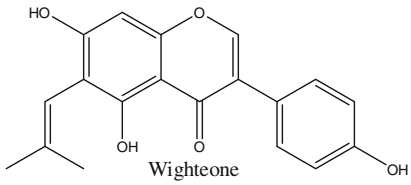
12.3.14 Erythrina variegata

Wighteone (an isoflavone) and oleanolic acid (a triterpenoid) have been isolated from stem bark extract of the plant (Xiaoli et al. 2006). Synthetic oleanolic acid as the aglycone form reportedly possessed fungicidal activity against *Sclerotina sclerotiorum*, *R. solani*, *B. cinerea*, and *P. parasitica* (Zhao et al. 2011). Oleanolic and ursolic acid, isolated from *L. salicaria* L. (Lythraceae) showed activity against the phytopathogenic fungus, *C. cucumerinum* (Becker et al. 2005). Oleanolic acid also showed the highest anti-fungal activity against *Saccharomyces carlsbergensis*

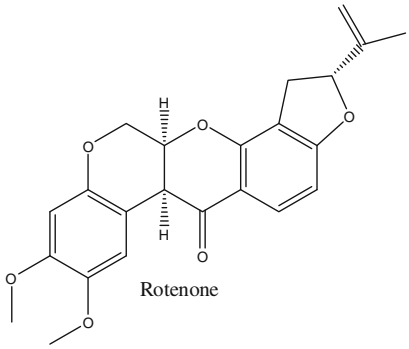
among 49 pentacyclic triterpenoids and their glycosides derived from plants or of semi-synthetic origin (Anisimov et al. 1979). Wighteone has been shown to possess excellent anti-fungal activity against *S. cerevisiae* (MIC value of 4 μg/ml). Wighteone, isolated from *Cudrania fruticosa* (Roxb.) Wight ex Kurz (Moraceae) also demonstrated anti-fungal activity against *C. albicans* (MIC value of 12.5 μg/ml) (Wang et al. 2005; Yin et al. 2006). The compound isolated from *C. cochinchinensis* (Lour.) Kudo. and Masam (Moraceae) exhibited anti-fungal activities against *C. neoformans*, *A. fumigatus,* and *A. nidulans* (MICs = 2–8 μg/ml) (Fukai et al. 2003).



Oleanolic acid



Wighteone



Rotenone

12.3.15 *Lablab purpureus*

Direct evidence for anti-fungal activity is not available for this plant. However, a lectin-like protein has been reported for the plant, which can inhibit *A. flavus* and fungal α-amylases (Fakhoury and Woloshuk 2001; Kim et al. 2007). Six rotenoids have been isolated from the plant with bioefficacy against malaria, dracunculiasis, and amoebiasis (Kamal and Mathur 2010). Rotenone has been observed to enhance the anti-fungal properties of staurosporine against *A. fumigatus*, *C. albicans*, and *Neurospora crassa* (Castro et al. 2010).

12.3.16 *Asparagus racemosus*

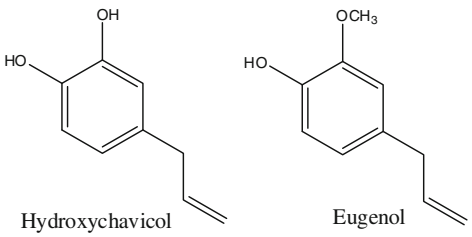
Extract of roots and tubers showed anti-fungal activity against a number of *Candida* species isolated from vaginal thrush patients like *C. albicans*, *C. tropicalis*, *C. krusei*, *C. guillermondii*, *C. parapsilosis*, and *C. stellatoida* (Uma et al. 2009). Crude ethanol extract of dry powdered roots showed a MIC value between 5 and 20 mg/ml for dermatopathogens (Potduang et al. 2008).

12.3.17 *Lawsonia inermis*

The plant is used by rural area patients of Turkey for treatment of tinea pedis infections (Kiraz et al. 2010). Dry leaves of the plant (also known as henna) reportedly showed in vitro anti-fungal activity against *C. albicans* (Habbal et al. 2005). Extract of bark of the plant showed broad fungitoxic spectrum when tested against 13 ringworm fungi, including *M. gypseum* and *T. mentagrophytes* (Singh and Pandey 1989). The fungitoxic principle from leaves of the plant appears to be lawsone (see Sect. 12.3.4).

12.3.18 *Piper betle*

Free and bound flavonoids of the plant have been reported to be most effective as an antidermatophytic against human pathogenic dermatophytes *T. rubrum*, *T. mentagrophytes*, *M. gypseum*, and *C. albicans* (Bhadauria and Kumar 2012).

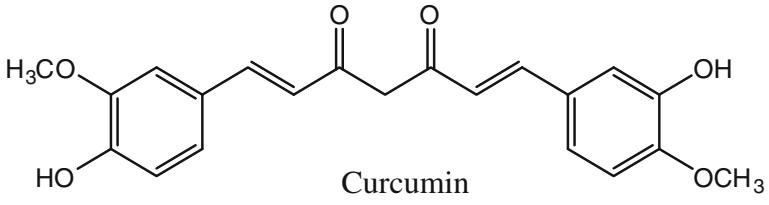


Eugenol and acetyleugenol have been identified as the major components of the essential oil obtained from the plant effective against *A. flavus* (Prakash et al. 2010). In vitro anti-fungal activity against various *Aspergillus* and *Candida* species was observed with hydroxychavicol, isolated from chloroform extraction of the aqueous leaf extract of the plant (Ali et al. 2010). Crude ethanolic extracts of leaves suppressed growth of *M. canis*, *M. gypseum*, and *T. mentagrophytes* with average IC_{50} values ranging from 110.44 to 119.00 μg/ml (Trakranrungsie et al. 2008).

The anti-fungal activity of eugenol has been well established. The compound has been reported to exhibit excellent fungicidal activity against pathogenic yeasts, including isolates resistant to azoles. In various *Candida* species, the anti-fungal mechanism has been attributed to effects on H(+)-ATPase mediated H(+)-pumping (Ahmad et al. 2010a). Against clinical *Candida* isolates, eugenol and methyleugenol have been shown to demonstrate synergistic action with fluconazole (Ahmad et al. 2010b; Khan and Ahmad 2012). Eugenol and carvacrol reportedly inhibited oral *Candida* strains isolated from denture wearers (Marcos-Arias et al. 2011). Eugenol has been demonstrated to inhibit fungal growth of *A. niger*, *A. terreus*, *Emericella nidulans*, *P. expansum*, *P. glabrum*, *P. italicum*, *F. oxysporum*, and *F. avenaceum* (Campaniello et al. 2010). The anti-fungal activity of eugenol against *Candida* isolates has been shown to increase with the addition of a methyl group to the compound (Ahmad et al. 2010c). Eugenol has further been shown to have in vitro inhibitory activity against *C. albicans* biofilms (He et al. 2007). In guinea pig models, eugenol isolated from Japanese cypress oil showed inhibitory activity against *M. gypseum* (Lee et al. 2007). Extract of *O. gratissimum* L. (Lamiaceae) has been found effective against *C. neoformans*; the active ingredient has been identified as eugenol (Lemos Jde et al. 2005). Carvacrol and eugenol have been showed efficacious as anti-fungal agents in the treatment of oral candidiasis induced by *C. albicans* in immunosuppressed rats (Chami et al. 2005).

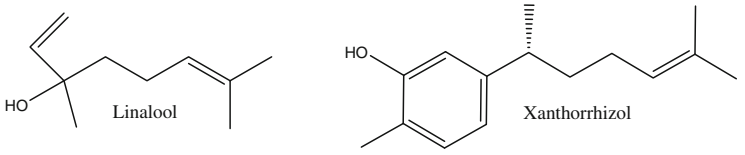
12.3.19 Curcuma aromatica

There is no direct evidence for anti-fungal activity of this plant. However, the plant reportedly contains constituents like sesquiterpene and curcuminoids (curcumin, demethoxycurcumin) (Bamba et al. 2011), which are reported to possess anti-fungal activities. A number of reports have mentioned the anti-candidal activity of curcumin, particularly against *C. albicans* (Dovigo et al. 2011a, b; Neelofar et al. 2011; Khan et al. 2012; Sharma et al. 2012). Tetrahydrocurcuminoids reportedly showed in vitro inhibitory effect on *F. proliferatum* growth and fumonisin B_1 biosynthesis (Coma et al. 2011). Curcumin has been shown to act alone or synergistically with azoles and polyenes to inhibit *C. albicans* through a mechanism involving generation of reactive oxygen species leading to apoptosis (Sharma et al. 2010a, b).



Curcumin

Other anti-fungal components isolated from essential oils from rhizomes of the plant cultivated in Japan include 1,8-cineole and linalool, whereas Indian cultivated plants showed presence of xanthorrhizol in the essential oil from rhizomes (Kojima et al. 1998). The anti-fungal properties of 1,8-cineole have been discussed in Sect. 12.3.12. Linalool, present in essential oil of *O. sanctum* L. (Lamiaceae), showed inhibitory activity against *Candida* species by disrupting ergosterol biosynthesis and fungal membrane integrity, as well as inhibition of H+ extrusion (Khan et al. 2010a, b). Linalool not only has been shown to inhibit *C. albicans*, but also along with fluconazole exhibited synergistic activity against a fluconazole-resistant strain of *C. albicans* (Zore et al. 2011). Linalool, isolated from essential oil of *L. angustifolia* Mill. (Lamiaceae), has also been reported to inhibit growth and hyphal elongation of *C. albicans* (D′Auria et al. 2005). Linalool, isolated from *Melaleuca alternifolia* (Maiden and Betche) Cheel (Myrtaceae) essential oil, has been reported to demonstrate anti-fungal activity against a range of fungi (Hammer et al. 2003).



Xanthorrhizol has been shown to demonstrate in vitro activity against *C. glabrata*, *C. guilliermondii*, and *C. parapsilosis* biofilms (Rukayadi et al. 2011). A synergistic anti-candidal activity of xanthorrhizol in combination with ketoconazole or amphotericin B has been observed against *C. albicans*, *C. glabrata*, *C. guilliermondii*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis* (Rukayadi et al. 2009). Xanthorrhizol isolated from *Curcuma xanthorrhiza* Roxb. (Zingiberaceae) reportedly inhibited the growth of *M.furfur* ATCC 14521 and *M.pachydermatis* ATCC 14522 (Rukayadi and Hwang 2007a) and opportunistic filamentous fungi like *A. flavus*, *A. fumigatus*, *A. niger*, *F. oxysporum*, *Rhizopus oryzae* and *T. mentagrophytes* (Rukayadi and Hwang 2007b), as well as various *Candida* species like *C. albicans*, *C. glabrata*, *C. guilliermondii*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis* (Rukayadi et al. 2006).

12.4 Conclusions

Our ethnomedicinal surveys among the traditional medical practitioners of Bangladesh have led to the enlisting of 26 local medicinal plants that haven proven benefit against tinea infection among the tribal and rural people. In this review, we carried out independent and the exhaustive literature mining with a view to finding any scientific rationale underlying such use of these plants. It is rather impressive and reassuring that few plants have exact use (i.e., against tinea) elsewhere in the world whilst many have proven global use against some forms of fungal infections.

What is most intriguing is that almost all the enlisted plants contain few or several chemical constituents that evidently possess antifungal activity when isolated from same or other plants in bioassay-guided way. We believe these findings will pave the way for further phyto-pharmacological characterization and development of more effective (both pharmacologically and cost wise) crude as well as modern medicinal preparations not only for tinea but also for other forms of common fungal infections.

12.5 Future Perspectives

Fungal infections including tinea are not easy to deal with (the problem often, in simple term, is nagging affecting the quality of life) and the situation is even worse for the immunocompromised (e.g. AIDS) and malnourished people. Although quite a few drugs are clinically available for treating systemic and topical fungal diseases, the efficacy in most cases is not that great and more importantly, most of them have limited spectrum and are fungistatic, not fungicidal. The latter requires prolong use of those drugs that not only frustrate patient compliance but more seriously can predispose to some unwanted effects due to their interactions with off-targets, other drugs, or food ingredients. The efficacy of these drugs may also diminish upon prolong use due to emergence of resistant fungal strains. The potential for developing cross-resistance is also not trivial. Therefore, the need for novel, efficacious, safer and broader spectrum antifungal agents can not be over emphasized. From this perspective, the above-mentioned medicinal plants may lend themselves to systematic modern scientific explorations in pursuit of novel antifungal agents. The fact that we need to know the identity and precise mechanism of action of specific antifungal constituent(s) will always remain as the priority. The medicinal chemists can then appreciate some chemical scaffolds common to these antifungal compounds and pursue detail structure–activity relationships with the lead compounds. The growing advancements in various in silico approaches (i.e., ligand-driven cheminformatic searches, computational docking against published 3D protein targets of fungal origin etc.) may aid such process.

References

Abraham A, Mohapatra LN, Kandhari KC, Pandhi RK, Bhutani LK (1975) The effects of some hair oils and unsaturated fatty acids on experimentally induced dermatophysis. Dermatologica 151:144–148

Abu-Taleb AM, El-Deeb K, Al-Otibi FO (2011) Assessment of antifungal activity of *Rumex vesicarius* L. and *Ziziphus spina-christi* (L.) Willd. extracts against two phytopathogenic fungi. Afr J Microbiol Res 5:1001–1011

Acharya TK, Chatterjee IB (1975) Isolation of chrysophanic acid-9-anthrone, the major antifungal principle of *Cassia tora*. Lloydia 38:218–220

Agarwal SK, Singh SS, Verma S, Kumar S (2000) Antifungal activity of anthraquinone derivatives of *Rheum emodi*. J Ethnopharmacol 72:43–46

Agnese AM, Pérez C, Cabrera JL (2001) *Adesmia aegiceras*: antimicrobial activity and chemical study. Phytomedicine 8:389–394

Agrawal A, Srivastava S, Srivastava JN, Srivastava MM (2004) Evaluation of inhibitory effect of the plant *Phyllanthus amarus* against dermatophytic fungi *Microsporum gypseum*. Biomed Environ Sci 17:359–365

Ahmad MU, Hai MA, Rahman MA (1998) Non-polar constituents of the leaves of *Ipomoea fistulosa*. J Bangladesh Acad Sci 22:167–171

Ahmad A, Khan A, Yousuf S, Khan LA, Manzoor N (2010a) Proton translocating ATPase mediated fungicidal activity of eugenol and thymol. Fitoterapia 81:1157–1162

Ahmad A, Khan A, Khan LA, Manzoor N (2010b) In vitro synergy of eugenol and methyleugenol with fluconazole against clinical *Candida* isolates. J Med Microbiol 59(Pt 10):1178–1184

Ahmad A, Khan A, Manzoor N, Khan LA (2010c) Evolution of ergosterol biosynthesis inhibitors as fungicidal against *Candida*. Microb Pathog 48:35–41

Ajose FO (2007) Some Nigerian plants of dermatologic importance. Int J Dermatol 46(Suppl 1):48–55

Ali MI, Shalaby NM, Elgamal MH, Mousa AS (1999) Antifungal effects of different plant extracts and their major components of selected aloe species. Phytother Res 13:401–407

Ali I, Khan FG, Suri KA, Gupta BD, Satti NK, Dutt P, Afrin F, Qazi GN, Khan IA (2010) In vitro antifungal activity of hydroxychavicol isolated from *Piper betle* L. Ann Clin Microbiol Antimicrob 9:7

Ambikapathy V, Gomathi S, Panneerselvam A (2011) Effect of antifungal activity of some medicinal plants against *Pythium debaryanum* (Hesse). Asian J Plant Sci Res 1:131–134

Anisimov MM, Shcheglov VV, Strigina LI, Chetyrina NS, Uvarova NI, Oshitok GI, Alad'ina NG, Vecherko LP, Zorina AD, Matyukhina LG, Saltykova IA (1979) Chemical structure and antifungal activity of a number of triterpenoids. Biol Bull Acad Sci USSR 6:464–468

Bamba Y, Yun S, Kunugi A, Inoue H (2011) Compounds isolated from *Curcuma aromatica* Salisb. Inhibit human P450 enzymes. J Nat Med 65:583–587

Becker H, Scher JM, Speakman JB, Zapp J (2005) Bioactivity guided isolation of antimicrobial compounds from *Lythrum salicaria*. Fitoterapia 76:580–584

Bernardes I, Felipe Rodrigues MP, Bacelli GK, Munin E, Alves LP, Costa MS (2012) *Aloe vera* extract reduces both growth and germ tube formation by *Candida albicans*. Mycoses 55:257–261

Bhadauria S, Kumar P (2012) Broad spectrum antidermatophytic drug for the control of tinea infection in human beings. Mycoses 55:339–343

Brusotti G, Cesari I, Gilardoni G, Tosi S, Grisoli P, Picco AM, Caccialanza G (2012) Chemical composition and antimicrobial activity of *Phyllanthus muellerianus* (Kuntze) Excell essential oil. J Ethnopharmacol 142:657–662

Campaniello D, Corbo MR, Sinigaglia M (2010) Antifungal activity of eugenol against *Penicillium*, *Aspergillus*, and *Fusarium* species. J Food Prot 73:1124–1128

Castro A, Lemos C, Falcão A, Fernandes AS, Glass NL, Videira A (2010) Rotenone enhances the antifungal properties of staurosporine. Eukaryot Cell 9:906–914

Cavaliero C, Gonçalves MJ, Serra D, Santoro G, Tomi F, Bighelli A, Salgueiro L, Casanova J (2011) Composition of a volatile extract of *Eryngium duriaei* subsp. *juresianum* (M. Laínz) M. Laínz, signalised by the antifungal activity. J Pharm Biomed Anal 54:619–622

Chaieb K, Zmantar T, Ksouri R, Hajlaoui H, Mahdouani K, Abdelly C, Bakhrouf A (2007) Antioxidant properties of the essential oil of *Eugenia caryophyllata* and its antifungal activity against a large number of clinical *Candida* species. Mycoses 50:403–406

Chami N, Bennis S, Chami F, Aboussekhra A, Remmal A (2005) Study of anticandidal activity of carvacrol and eugenol in vitro and in vivo. Oral Microbiol Immunol 20:106–111

Chen YT, Hsu LH, Huang IP, Tsai TC, Lee GC, Shaw JF (2007) Gene cloning and characterization of a novel recombinant antifungal chitinase from papaya (*Carica papaya*). J Agric Food Chem 55:714–722

Coma V, Portes E, Gardrat C, Richard-Forget F, Castellan A (2011) In vitro inhibitory effect of tetrahydrocurcuminoids on *Fusarium proliferatum* growth and fumonisin B_1 biosynthesis. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 28:218–225

Crockett CO, Guede-Guina F, Pugh D, Vangah-Manda M, Robinson TJ, Olubadewo JO, Ochillo RF (1992) *Cassia alata* and the preclinical serach for therapeutic agents for the treatment of opportunistic infections in AIDS patients. Cell Mol Biol (Noisy-le-grand) 38:799–802

Cruz EA, Da-Silva SA, Muzitano MF, Silva PM, Costa SS, Rossi-Bergmann B (2008) Immunomodulatory pretreatment with *Kalanchoe pinnata* extract and its quercitrin flavonoid effectively protects mice against fatal anaphylactic shock. Int Immunopharmacol 8:1616–1621

Cruz EA, Reuter S, Martin H, Dehzad N, Muzitano MF, Costa SS, Rossi-Bergmann B, Buhl R, Stassen M, Taube C (2012) *Kalanchoe pinnata* inhibits mast cell activation and prevents allergic airway disease. Phytomedicine 19:115–121

Dambolena JS, López AG, Cánepa MC, Theumer MG, Zygadlo JA, Rubinstein HR (2008) Inhibitory effects of cyclic terpenes (limonene, menthol, menthone athymol) on *Fusarium verticillioides* MRC 826 growth and fumonisin B_1 biosynthesis. Toxicon 51:37–44

Damodaran S, Venkataraman S (1994) A study on the therapeutic efficacy of *Cassia alata* Linn. leaf extract against *Pityriasis versicolor*. J Ethnopharmacol 42:19–23

Daneshtalab M (2008) Discovery of chlorogenic acid-based peptidomimetics as a novel class of antifungals. A success story in rational drug design. J Pharm Pharm Sci 11:44s–55s

Das S, Mishra B, Gill K, Ashraf MS, Singh AK, Sinha M, Sharma S, Xess I, Dalal K, Singh TP, Dey S (2011) Isolation and characterization of novel protein with anti-fungal and anti-inflammatory properties from *Aloe vera* leaf gel. Int J Biol Macromol 48:38–43

D′Auria FD, Tecca M, Strippoli V, Salvatore G, Battinelli L, Mazzanti G (2005) Antifungal activity of Lavandula angustifolia essential oil against *Candida albicans* yeast and mycelia form. Med Mycol 43:391–396

DebMandal M, Mandal S (2011) Coconut (*Cocos nucifera* L.: Arecaceae): in health promotion and disease prevention. Asian Pac J Trop Med 4:241–247

Dharmaratne HR, Napagoda MT, Tennakoon SB (2009) Xanthones from roots of *Calophyllum thwaitesii* and their bioactivity. Nat Prod Res 23:539–545

Dovigo LN, Pavarina AC, Carmello JC, Machado AL, Brunetti IL, Bagnato VS (2011a) Susceptibility of clinical isolates of *Candida* to photodynamic effects of curcumin. Lasers Surg Med 43:927–934

Dovigo LN, Pavarina AC, Ribeiro AP, Brunetti IL, Costa CA, Jacomassi DP, Bagnato VS, Kurachi C (2011b) Investigation of the photodynamic effects of curcumin against *Candida albicans*. Photochem Photobiol 87:895–903

Duraipandiyan V, Ignacimuthu S (2007) Antibacterial and antifungal activity of *Cassia fistula* L.: an ethnomedicinal plant. J Ethnopharmacol 112:590–594

Duraipandiyan V, Gnanasekar M, Ignacimuthu S (2010) Antifungal activity of triterpenoid isolated from *Azima tetracantha* leaves. Folia Histochem Cytobiol 48:311–313

Fakhoury AM, Woloshuk CP (2001) Inhibition of growth of *Aspergillus flavus* and fungal α-amylases by a lectin like protein from *Lablab purpureus*. Mol Plant Microbe Interact 14:955–961

Fernand VE, Dinh DT, Washington SJ, Fakayode SO, Losso JN, van Ravenswaay RO, Warner IM (2008) Determination of pharmacologically active compounds in root extracts of *Cassia alata* L. by use of high performance liquid chromatography. Talanta 74:896–902

Fernández-Ocaña AM, Gómez-Rodríguez MV, Velasco-Negueruela A, Camacho-Simarro AM, Fernández-López C, Altarejos J (2004) In vivo antifungal activity of the essential oil of *Bupleurum gibraltarium* against *Plasmopara halstedii* in sunflower. J Agric Food Chem 52:6414–6417

Fukai T, Yonekawa M, Hou AJ, Nomura T, Sun HD, Uno J (2003) Antifungal agents from the roots of *Cudrania cochinchinensis* against *Candida*, *Cryptococcus*, and *Aspergillus* species. J Nat Prod 66:1118–1120

Gertsch J, Tobler RT, Brun R, Sticher O, Heilmann J (2003) Antifungal, antiprotozoal, cytotoxic and piscicidal properties of Justicidin B and a new arylnaphthalide lignan from *Phyllanthus piscatorum*. Planta Med 69:420–424

Giordani R, Siepaio M, Moulin-Traffort J, Régli P (1991) Antifungal action of *Carica papaya* latex: isolation of fungal cell wall hydrolyzing enzymes. Mycoses 34:469–477

Giordani R, Cardenas ML, Moulin-Traffort J, Régli P (1996) Fungicidal activity of latex sap from *Carica papaya* and antifungal effect of D(+)-glucosamine on *Candida albicans* growth. Mycoses 39:103–110

Giordani R, Gachon C, Moulin-Traffort J, Régli P (1997) A synergistic effect of *Carica papaya* latex sap and fluconazole on *Candida albicans* growth. Mycoses 40:429–437

Gopalakrishnan C, Shankaranarayanan D, Nazimudeen SK, Viswanathan S, kameswaran L (1980) Antiinflammatory and CNS depressant activities of xanthones from *Calophyllum inophyllum* and *Mesua ferrea*. Ind J Pharmacol 12:181–191

Goun E, Cunningham G, Chu D, Nguyen C, Miles D (2003) Antibacterial and antifungal activity of Indonesian ethnomedicinal plants. Fitoterapia 74:592–596

Habbal OA, Al-Jabri AA, El-Hag AH, Al-Mahrooqi ZH, Al-Hashmi NA (2005) *In-vitro* antimicrobial activity of *Lawsonia inermis* Linn (henna). A pilot study of the Omani henna. Saudi Med J 26:69–72

Hammer KA, Carson CF, Riley TV (2003) Antifungal activity of the components of *Melaleuca alternifolia* (tea tree) oil. J Appl Microbiol 95:853–860

Hammerschmidt FJ, Clark AM, Soliman FM, el-Kashoury ES, Abd el-Kawy MM, el-Fishawy AM (1993) Chemical composition and antimicrobial activity of essential oils of Jasonia candicans and Jasonia montana. Planta Med 59:68–70

Hazni H, Ahmad N, Hitotsuyanagi Y, Takeya K, Choo CY (2008) Phytochemical constituents from *Cassia alata* with inhibition against methicillin-resistant *Staphylococcus aureus* (MRSA). Planta Med 74:1802–1805

He M, Du M, Fan M, Bian Z (2007) *In vitro* activity of eugenol against *Candida albicans* biofilms. Mycopathologia 163:137–143

Hong EJ, Na KJ, Choi IG, Choi KC, Jeung EB (2004) Antibacterial and antifungal effects of essential oils from coniferous trees. Biol Pharm Bull 27:863–866

Ibrahim D, Osman H (1995) Antimicrobial activity of *Cassia alata* from Malaysia. J Ethnopharmacol 45:151–156

Jabeen K, Javaid A, Ahmad E, Athar M (2011) Antifungal compounds from *Melia azedarach* leaves for management of *Ascochyta rabiei*, the cause of chickpea blight. Nat Prod Res 25:264–276

Jain R, Chitale G, Sharma P, Jain SC (2010) Phytochemical and antimicrobial investigations of *Cassia alata* Linn. roots. J Med Arom Plant Sci 32:13–15

Jang DS, Lee GY, Kim YS, Lee YM, Kim CS, Yoo JL, Kim JS (2007) Anthraquinones from the seeds of *Cassia tora* with inhibitory activity on protein glycation and aldose reductase. Biol Pharm Bull 30:2207–2210

Jardim CM, Jham GN, Dhingra OD, Freire MM (2008) Composition and antifungal activity of the essential oil of the Brazilian *Chenopodium ambrosioides* L. J Chem Ecol 34:1213–1218

Jasicka-Misiak I, Lipok J, Swider IA, Kafarski P (2010) Possible fungistatic implications of betulin presence in betulaceae plants and their hymenochaetaceae parasitic fungi. Z Naturforsch C 65:201–206

Javaid A, Amin M (2009) Antifungal activity of methanol and n-hexane extracts of three *Chenopodium* species against *Macrophomina phaseolina*. Nat Prod Res 23:1120–1127

Kamal R, Mathur N (2010) Rotenoids from *Lablab purpureus* L. and their bioefficacy against human disease vectors. Parasitol Res 107:1481–1488

Kang K, Fong WP, Tsang PW (2010) Novel antifungal activity of purpurin against *Candida* species *in vitro*. Med Mycol 48:904–911

Kanwal Q, Hussain I, Latif Siddiqui H, Javaid A (2010) Antifungal activity of flavonoids isolated from mango (*Mangifera indica* L.) leaves. Nat Prod Res 24:1907–1914

Kaomongkolgit R, Jamdee K, Chaisomboon N (2009) Antifungal activity of α-mangostin against *Candida albicans*. J Oral Sci 51:401–406

Khan A, Ahmad A, Akhtar F, Yousuf S, Xess I, Khan LA, Manzoor N (2010a) *Ocimum sanctum* essential oil and its active principles exert their antifungal activity by disrupting ergosterol biosynthesis and membrane integrity. Res Microbiol 161:816–823

Khan A, Ahmad A, Manzoor N, Khan LA (2010b) Antifungal activities of *Ocimum sanctum* essential oil and its lead molecules. Nat Prod Commun 5:345–349

Khan MS, Ahmad I (2012) Antibiofilm activity of certain phytocompounds and their synergy with fluconazole against *Candida albicans* biofilms. J Antimicrob Chemother 67:618–621

Khan N, Shreaz S, Bhatia R, Ahmad SI, Muralidhar S, Manzoor N, Khan LA (2012) Anticandidal activity of curcumin and methyl cinnamaldehyde. Fitoterapia 83:434–440

Kim YH, Woloshuk CP, Cho EH, Bae JM, Song YS, Huh GH (2007) Cloning and functional expression of the gene encoding an inhibitor against *Aspergillus flavus* α-amylase, a novel seed lectin from *Lablab purpureus* (*Dolichos lablab*). Plant Cell Rep 26:395–405

Kim YM, Lee CH, Kim HG, Lee HS (2004) Anthraquinones isolated from *Cassia tora* (Leguminosae) seed show an antifungal property against phytopathogenic fungi. J Agric Food Chem 52:6096–6100

Kiraz N, Metintas S, Oz Y, Koc F, Koku Aksu EA, Kalyoncu C, Kasifoglu N, Cetin E, Arikan I (2010) The prevalence of tinea pedis and tinea manuum in adults in rural areas in Turkey. Int J Environ Health Res 20:379–386

Kishore N, Mishra AK, Chansouria JP (1993) Fungitoxicity of essential oils against dermatophytes. Mycoses 36:211–215

Kojima H, Yanai T, Toyota A (1998) Essential oil constituents from Japanese and Indian *Curcuma aromatica* rhizomes. Planta Med 64:380–381

Kordali S, Cakir A, Ozer H, Cakmakci R, Kesdek M, Mete E (2008) Antifungal, phytotoxic and insecticidal properties of essential oil isolated from Turkish *Origanum acutidens* and its three components, carvacrol, thymol and p-cymene. Bioresour Technol 99:8788–8795

Kuiate JR, Mouokeu S, Wabo HK, Tane P (2007) Antidermatophytic triterpenoids from *Syzygium jambos* (L.) Alston (Myrtaceae). Phytother Res 21:149–152

Kumar R, Mishra AK, Dubey NK, Tripathi YB (2007) Evaluation of *Chenopodium ambrosioides* oil as a potential source of antifungal, antiaflatoxigenic and antioxidant activity. Int J Food Microbiol 115:159–164

Kwon HJ, Hong YK, park C, Choi YH, Yun HJ, Lee EW, Kim BW (2010a) Widdrol induces cell cycle arrest, associated with MCM down-regulation, in human colon adenocarcinoma cells. Cancer Lett 290:96–103

Kwon HJ, Lee EW, Hong YK, Yun HJ, Kim BW (2010b) Widdrol from *Juniperus chinensis* induces apoptosis in human colon adenocarcinoma HT29 cells. Biotechnol Bioprocess Eng (BBE) 15:167–172

Lamidi M, Rondi ML, Ollivier E, Faure R, Ekekang L, Nze Balansard G (2000) Constituents of *Ipomoea fistulosa* leaves. Fitoterapia 71:203–204

Lee SJ, Han JI, Lee GS, Park MJ, Choi IG, Na KJ, Jeung EB (2007) Antifungal effect of eugenol and nerolidol against *Microsporum gypseum* in a guinea pig model. Biol Pharm Bull 30:184–188

Lee JH, Park JH, Kim YS, Han Y (2008) Chlorogenic acid, a polyphenolic compound, treats mice with septic arthritis caused by *Candida albicans*. Int Immunopharmacol 8:1681–1685

Lee CH, Park JM, Song HY, Jeong EY, Lee HS (2009) Acaricidal activities of major constituents of essential oil of *Juniperus chinensis* leaves against house dust and stored food mites. J Food Prot 72:1686–1691

Lemos Jde A, Passos XS, Fernandes Ode F, Paula JR, Ferri PH, Souza LK, Lemos Ade A, Silva Mdo R (2005) Antifungal activity from *Ocimum gratissimum* L. towards *Cryptococcus neoformans*. Mem Inst Oswaldo Cruz 100:55–58

Lim JP, Song YC, Kim JW, Ku CH, Eun JS, Leem KH, Kim DK (2002) Free radical scavengers from the heartwood of *Juniperus chinensis*. Arch Pharm Res 25:449–452

Liu X, Zhao M, Luo W, Yang B, Jiang Y (2009) Identification of volatile components in *Phyllanthus emblica* L. and their antimicrobial activity. J Med Food 12:423–428

Liu Y-H , Zhang Z, Shi G-X, Meng J-C, Tan R-X (2002) A new antifungal flavonol glycoside from *Hypericum perforatum*. Acta Botan Sin 44:743–745

Ma CM, Kully M, Khan JK, Hattori M, Daneshtalab M (2007) Synthesis of chlorogenic acid derivatives with promising antifungal activity. Bioorg Med Chem 15:6830–6833

Machado KE, Cechinel Filho V, Cruz RC, Meyre-Silva C, Cruz AB (2009) Antifungal activity of *Eugenia umbelliflora* against dermatophytes. Nat Prod Commun 4:1181–1184

Manojlovic NT, Solujic S, Sukdolak S, Milosev M (2005) Antifungal activity of *Rubia tinctorum*, *Rhamnus frangula* and *Caloplaca cerina*. Fitoterapia 76:244–246

Marcos-Arias C, Eraso E, Madariaga L, Quindós G (2011) In vitro activities of natural products against oral *Candida* isolates from denture wearers. BMC Complement Altern Med 11:119

Mishra BB, Kishore N, Tiwari VK, Singh DD, Tripathi V (2010) A novel antifungal anthraquinone from seeds of *Aegle marmelos* Correa (family Rutaceae). Fitoterapia 81:104–107

Morel C, Séraphin D, Teyrouz A, Larcher G, Bouchara JP, Litaudon M, Richomme P, Bruneton J (2002) New and antifungal xanthones from *Calophyllum caledonicum*. Planta Med 68:41–44

Muhammad N, Kamal M, Islam T, Islam N, Shafiquzzaman M (2009) A study to evaluate the efficacy and safety of oral fluconazole in the treatment of tinea versicolor. Mymensingh Med J 18:31–35

Muzitano MF, Cruz EA, de Almeida AP, Da Silva SA, Kaiser CR, Guette C, Rossi-Bergmann B, Costa SS (2006) Quercitrin: an antileishmanial flavonoid glycoside from *Kalanchoe pinnata*. Plant Med 72:81–83

Muzitano MF, Bergonzi MC, De Melo GO, Lage CL, Bilia AR, Vincieri FF, Rossi-Bergmann B, Costa SS (2011) Influence of cultivation conditions, season of collection and extraction method on the content of antileishmanial flavonoids from *Kalanchoe pinnata*. J Ethnopharmacol 133:132–137

Neelofar K, Shreaz S, Rimple B, Muralidhar S, Nikhat M, Khan LA (2011) Curcumin as a promising anticandidal of clinical interest. Can J Microbiol 57:204–210

Nuñez YO, Salabarria IS, Collado IG, Hernandez-Galan R (2006) The antifungal activity of widdrol and its biotransformation by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. and *Botrytis cinerea* Pers.: Fr. J Agric Food Chem 54:7517–7521

Nuñez YO, Salabarria IS, Collado IG, Hernández-Gálan R (2007) Sesquiterpenes from the wood of *Juniperus lucayana*. Phytochemistry 68:2409–2414

Ogunwande IA, Flamini G, Cioni PL, Omikorede O, Azeez RA, Ayodele AA, Kamil YO (2010) Aromatic plants growing in Nigeria: essential oil constituents of *Cassia alata* (Linn.) Roxb. and *Helianthus annuus* L. Rec Nat Prod 4:211–217

Ohashi H, Asai T, Kawai S (1994) Screening of main Japanese conifers for antifungal leaf components. Sesquiterpenes of Juniperus chinensis pyramidalis. Holzforschung 48:193–198

Oku H, Ishiguro K (2001) Antipruritic and antidermatitic effect of extract and compounds of *Impatiens balsamina* L. in atopic dermatitis model of NC mice. Phytother Res 15:506–510

Okwu DE, Nnamdi FU (2011a) A novel antimicrobial phenanthrene alkaloid from *Bryophyllum pinnatum*. J Chem Pharmaceut Res 3:27–33

Okwu DE, Nnamdi FU (2011b) Cannabinoid dronabinol alkaloid with antimicrobial activity from *Cassia alata* Linn. Chemica Sinica 2:247–254

Ozcan MM, Chalchat JC (2008) Chemical composition and antifungal activity of rosemary (*Rosmarinus officinalis* L.) oil from Turkey. Int J Food Sci Nutr 59:691–698

Palá-Paúl J, Usano-Alemany J, Granda E, Soria AC (2009) Chemical composition, antifungal and antibacterial activity of the essential oil of *Chamaecyparis nootkatensis* from Spain. Nat Prod Commun 4:1007–1010

Pattnaik S, Subramanyam VR, Bapaji M, Kole CR (1997) Antibacterial and antifungal activity of aromatic constituents of essential oils. Microbios 89:39–46

Pina-Vaz C, Gonçalves Rodrigues A, Pinto E, Costa-de-Oliveira S, Tavares C, Salgueiro L, Cavaleiro C, Gonçalves MJ, Martinez-de-Oliveira J (2004) Antifungal activity of *Thymus* oils and their major compounds. J Eur Acad Dermatol Venereol 18:73–78

Ponnusamy K, Petchiammal C, Mohankumar R, Hopper W (2010) In vitro antifungal activity of indirubin isolated from a South Indian ethnomedicinal plant *Wrightia tinctoria* R. Br. J Ethnopharmacol 132:349–354

Potduang B, Meeploy M, Giwanon R, Benmart Y, Kaewduang M, Supatanakul W (2008) Biological activities of *Asparagus racemosus*. Afr J Tradit Complement Altern Med 5:230–237

Prakash B, Shukla R, Singh P, Kumar A, Mishra PK, Dubey NK (2010) Efficacy of chemically characterized *Piper betle* L. essential oil against fungal and aflatoxin contamination of some edible commodities and its antioxidant activity. Int J Food Microbiol 142:114–119

Prasad CS, Shukla R, Kumar A, Dubey NK (2010) *In vitro* and *in vivo* antifungal activity of essential oils of *Cymbopogon martini* and *Chenopodium ambrosioides* and their synergism against dermatophytes. Mycoses 53:123–129

Rahman MS, Hasan AJMM, Ali MY, Ali MU (2006) Studies on the isolation of parahydroxy benzoic acid from the leaves of *Cassia alata*. Bangladesh J Sci Ind Res 41:89–92

Rahmatullah M, Ferdausi D, Mollik MAH, Jahan R, Chowdhury MH, Haque WM (2010a) A survey of medicinal plants used by Kavirajes of Chalna area, Khulna district, Bangladesh. Afr J Tradit Complement Altern Med 7:91–97

Rahmatullah M, Khatun MA, Morshed N, Neogi PK, Khan SUA, Hossan MS, Mahal MJ, Jahan R (2010b) A randomized survey of medicinal plants used by flok medicinal healers of Sylhet Division, Bangladesh. Adv Nat Appl Sci 4:52–62

Rahmatullah M, Kabir AABT, Rahman MM, Hossan MS, Khatun Z, Khatun MA, Jahan R (2010c) Ethnomedicinal practices among a minority group of Christians residing in Mirzapur village of Dinajpur District, Bangladesh. Adv Nat Appl Sci 4:45–51

Rahmatullah M, Momen MA, Rahman MM, Nasrin D, Hossain MS, Khatun Z, Jahan FI, Khatun MA, Jahan R (2010d) A randomized survey of medicinal plants used by folk medicinal practitioners in Daudkandi sub-district of Comilla district, Bangladesh. Adv Nat Appl Sci 4:99–104

Rahmatullah M, Mollik MAH, Ahmed MN, Bhuiyan MZA, Hossain MM, Azam MNK, Seraj S, Chowdhury MH, Jamal F, Ahsan S, Jahan R (2010e) A survey of medicinal plants used by folk medicinal practitioners in two villages of Tangail district, Bangladesh. Am.-Eur J Sustain Agric 4:357–362

Rahmatullah M, Mollik MAH, Islam MK, Islam MR, Jahan FI, Khatun Z, Seraj S, Chowdhury MH, Islam F, Miajee ZUM, Jahan R (2010f) A survey of medicinal and functional food plants used by the folk medicinal practitioners of three villages in Sreepur Upazilla, Magura district, Bangladesh. Am.-Eur J Sustain Agric 4:363–373

Rahmatullah M, Jahan R, Khatun MA, Jahan FI, Azad AK, Bashar ABM, Miajee ZUM, Ahsan S, Nahar N, Ahmad I, Chowdhury MH (2010g) A pharmacological evaluation of medicinal plants used by folk medicinal practitioners of Station Purbo Para Village of Jamalpur Sadar Upazila in Jamalpur district, Bangladesh. Am.-Eur J Sustain Agric 4:170–195

Rahmatullah M, Biswas KR (2012) Traditional medicinal practices of a sardar healer of the Sardar (Dhangor) community of Bangladesh. J Altern Complement Med 18:10–19

Rahmatullah M, Hasan A, Parvin W, Moniruzzaman M, Khatun A, Khatun Z, Jahan FI, Jahan R (2012a) Medicinal plants and formulations used by the Soren clan of the Santal tribe in Rajshahi district, Bangladesh for treatment of various ailments. Afr J Tradit Complement Altern Med 9:342–349

Rahmatullah M, Khatun Z, Hasan A, Parvin W, Moniruzzaman M, Khatun A, Mahal MJ, Bhuiyan MSA, Mou SM, Jahan R (2012b) Survey and scientific evaluation of medicinal plants used by the Pahan and Teli tribal communities of Natore district, Bangladesh. Afr J Tradit Complement Altern Med 9:366–373

Rahmatullah M, Azam MNK, Khatun Z, Seraj S, Islam F, Rahman MA, Jahan S, Aziz MS, Jahan R (2012c) Medicinal plants used for treatment of diabetes by the Marakh sect of the Garo tribe living in Mymensingh district, Bangladesh. Afr J Tradit Complement Altern Med 9:380–385

Raina VK, Srivastava SK, Syamsundar KV (2005) Essential oil composition of *Juniperus chinensis* from the plains of northern India. Flavour Frag J 20:57–59

Ranganathan S, Balajee SA (2000) Anti-*Cryptococcus* activity of combination of extracts of *Cassia alata* and *Ocimum sanctum*. Mycoses 43:299–301

Rocha AD, de Oliveira AB, de Souza Filho JD, Lombardi JA, Braga FC (2004) Antifungal constituents of *Clytostoma ramentaceum* and *Mansoa hirsuta*. Phytother Res 18:463–467

Romagnoli C, Andreotti E, Maietti S, Mahendra R, Mares D (2010) Antifungal activity of essential oil from fruits of Indian *Cuminum cyminum*. Pharm Biol 48:834–838

Rosca-Casian O, Parvu M, Vlase L, Tamas M (2007) Antifungal activity of *Aloe vera* leaves. Fitoterapia 78:219–222

Rukayadi Y, Yong D, Hwang JK (2006) In vitro anticandidal activity of xanthorrhizol isolated from *Curcuma xanthorrhiza* Roxb. J Antimicrob Chemother 57:1231–1234

Rukayadi Y, Hwang JK (2007a) In vitro anti-*Malassezia* activity of xanthorrhizol isolated from *Curcuma xanthorrhiza* Roxb. Lett Appl Microbiol 44:126–130

Rukayadi Y, Hwang JK (2007b) In vitro antimycotic activity of xanthorrhizol isolated from *Curcuma xanthorrhiza* Roxb. against opportunistic filamentous fungi. Phytother Res 21:434–438

Rukayadi Y, Lee K, Lee MS, Yong D, Hwang JK (2009) Synergistic anticandidal activity of xanthorrhizol in combination with ketoconazole or amphotericin B. FEMS Yeast Res 9:1302–1311

Rukayadi Y, Han S, Yong D, Hwang JK (2011) *In vitro* activity of xanthorrhizol against *Candida glabrata*, *C. guilliermondii*, and *C. parapsilosis* biofilms. Med Mycol 49:1–9

Sabulal B, Dan M, J AJ, Kurup R, Pradeep NS, Valsamma RK, George V (2006) Caryophyllene-rich rhizome oil of *Zingiber nimmonii* from South India: Chemical characterization and antimicrobial activity. Phytochemistry 67:2469–2473

Sadeque JB, Shahidullah M, Shah OR, Kamal M (1995) Systemic ketoconazole in the treatment of tinea versicolor. Int J Dermatol 34:504–505

Safaei-Ghomi J, Ahd AA (2010) Antimicrobial and antifungal properties of the essential oil and methanol extracts of *Eucalyptus largiflorens* and *Eucalyptus intertexta*. Pharmacogn Mag 6:172–175

Sakunphueak A, Panichayupakaranant P (2012) Comparison of antimicrobial activities of naphthoquinones from *Impatiens balsamina*. Nat Prod Res 26:1119–1124

Sattar EA, Gala A, Rashwan O (1995) Caffeoyl derivatives from the seeds of *Ipomoea fistulosa*. Int J Pharmacognosy 33:155–158

Sekine T, Sugano M, Majid A, Fujii Y (2007) Antifungal effects of volatile compounds from black zira (*Bunium persicum*) and other spices and herbs. J Chem Ecol 33:2123–2132

Shah VH, Mehta DS (2006) Phytochemical investigation of *Ipomoea fistulosa*, Jacq.: part I—isolation and structure determination of an alkaloid—ergosine. Chemistry: Indian J 3:8–10

Shai LJ, McGaw LJ, Aderogba MA, Mdee LK, Eloff JN (2008) Four pentacyclic triterpenoids with antifungal and antibacterial activity from *Curtisia dentata* (Burm.f) C.A. Sm Leave J Ethnopharmacol 119:238–244

Shanker KS, Kanjilal S, Rao BV, Kishore KH, Misra S, Prasad RB (2007) Isolation and antimicrobial evaluation of isomeric hydroxyl ketones in leaf cuticular waxes of *Annona squamosa*. Phytochem Anal 18:7–12

Sharma M, Manoharlal R, Puri N, Prasad R (2010a) Antifungal curcumin induces reactive oxygen species and triggers an early apoptosis but prevents hyphae development by targeting the global repressor TUP1 in *Candida albicans*. Biosci Rep 30:391–404

Sharma M, Manoharlal R, Negi AS, Prasad R (2010b) Synergistic anticandidal activity of pure polyphenol curcumin I in combination with azoles and polyenes generates reactive oxygen species leading to apoptosis. FEMS Yeast Res 10:570–578

Sharma M, Dhamgaye S, Singh A, Prasad R (2012) Lipidome analysis reveals antifungal polyphenol curcumin affects membrane lipid homeostasis. Front Biosci (Elite Ed) 4:1195–1209

Shikha P, Latha PG, Suja SR, Anuja GI, Shyamal S, Shine VJ, Sini S, Krishna Kumar NM, Rajasekharan S (2010) Anti-inflammatory and antinociceptive activity of *Justicia gendarussa* Burm.f. leaves. IJNPR 1:456–461

Singh O, Ali M, Akhtar N (2011) New antifungal xanthones from the seeds of *Rhus coriaria* L. Z Naturforsch C 66:17–23

Singh RK (2005) Fungitoxicity of some higher plants and synergistic activity of their essential oils against *Sclerotium rolfsii* Sacc. causing foot-rot disease of barley. Hindustan Antibiot Bull 47–48:45–51

Singh VK, Pandey DK (1989) Fungitoxic studies on bark extract of *Lawsonia inermis* against ringworm fungi. Hindustan Antibiot Bull 31:32–35

Soković MD, Vukojević J, Marin PD, Brkić DD, Vajs V, van Griensven LJ (2009) Chemical composition of essential oils of *Thymus* and *Mentha* species and their antifungal activities. Molecules 14:238–249

Somchit MN, Reezal I, Nur IE, Mutalib AR (2003) In vitro antimicrobial activity of ethanol and water extracts of *Cassia alata*. J Ethnopharmacol 84:1–4

Sritrairat N, Nukul N, Inthasama P, Sansuk A, Prasirt J, Leewatthanakorn T, Piamsawad U, Dejrudee A, Panichayupakaranant P, Pangsomboon K, Chanowanna N, Hintao J, Teanpaisan R, Chaethong W, Yongstar P, Pruphetkaew N, Chongsuvivatwong V, Nittayananta W (2011) Antifungal activity of lawsone methyl ether in comparison with chlorhexidine. J Oral Pathol Med 40:90–96

Teh SS, Cheng Lian Ee G, Rahmani M, Taufiq-Yap YH, Go R, Mah SH (2011) Pyranoxanthones from *Mesua ferrea*. Molecules 16:5647–5654

Thevissen K, François IE, Sijtsma L, van Amerongen A, Schaaper WM, Meloen R, Posthuma-Trumpie T, Broekaert WF, Cammue BP (2005) Antifungal activity of synthetic peptides derived from *Impatiens balsamina* antimicrobial peptides Ib-AMP1 and Ib-AMP4. Peptides 26:1113–1119

Tocci N, Simonetti G, D'Auria FD, Panella S, Palamara AT, Valletta A, Pasqua G (2011) Root cultures of *Hypericum perforatum* subsp. *angustifolium* elicited with chitosan and production of xanthone-rich extracts with antifungal activity. Appl Microbiol Biotechnol 91:977–987

Trakranrungsie N, Chatchawanchonteera A, Khunkitti W (2008) Ethnoveterinary study for antidermatophytic activity of *Piper betle*, *Alpinia galanga* and *Allium ascalonicum* extracts *in vitro*. Res Vet Sci 84:80–84

Tripathi RD, Srivastava HS, Dixit SN (1978) A fungitoxic principle from the leaves of *Lawsonia inermis* Lam. Experientia 34:51–52

Tuntiwachwuttikul P, Butsuri Y, Sukkoet P, Prawat U, Taylor WC (2008) Anthraquinones from the roots of *Prismatomeris malayana*. Nat Prod Res 22:962–968

Uma B, Prabhakar K, Rajendran S (2009) Anticandidal activity of *Asparagus racemosus*. Indian J Pharm Sci 71:342–343

Vale-Silva LA, Gonçalves MJ, Cavaleiro C, Salgueiro L, Pinto E (2010) Antifungal activity of the essential oil of *Thymus* x *viciosoi* against *Candida*, *Cryptococcus*, *Aspergillus* and dermatophyte species. Planta Med 76:882–888

Venkataraman S, Ramanujam TR, Venkatasubbu VS (1980) Antifungal activity of the alcoholic extract of coconut shell—*Cocos nucifera* Linn. J Ethnopharmacol 2:291–293

Villaroya MLE, Bernal-Santos R (1976) A chemical investigation of *Cassia alata* L. (fam. Leguminosae). Asian J Pharm 3:10–24

Villaseñor IM, Canlas AP, Pascua MP, Sabando MN, Soliven LA (2002) Bioactivity studies on *Cassia alata* Linn. leaf extracts. Phytother Res 16(Suppl 1):S93–96

Wang HX, Ng TB (2005) An antifungal peptide from the coconut. Peptides 26:2392–2396

Wang YH, Hou AJ, Zhu GF, Chen DF, Sun HD (2005) Cytotoxic and antifungal isoprenylated xanthones and flavonoids from *Cudrania fruticosa*. Planta Med 71:273–274

Xiaoli L, Naili W, Sau WM, Chen AS, Xinsheng Y (2006) Four new isoflavonoids from the stem bark of *Erythrina variegata*. Chem Pharm Bull (Tokyo) 54:570–573

Yin H, Zhao H, Zhang Y, Zhang H, Xu L, Zou Z, Yang W, Cheng J, Zhou Y (2006) Genome-wide analysis of the expression profile of *Saccharomyces cerevisiae* in response to treatment with the plant isoflavone, wighteone, as a potential antifungal agent. Biotechnol Lett 28:99–105

Yordanov M, Dimitrova P, Patkar S, Saso L, Ivanovska N (2008) Inhibition of *Candida albicans* extracellular enzyme activity by selected natural substances and their application in *Candida* infection. Can J Microbiol 54:435–440

Zhao H, Zong G, Zhang J, Wang D, Liang X (2011) Synthesis and anti-fungal activity of several oleanolic acid glycosides. Molecules 16:1113–1128

Zore GB, Thakre AD, Jadhav S, Karuppayil SM (2011) Terpenoids inhibit *Candida albicans* growth by affecting membrane integrity and the arrest of cell cycle. Phytomedicine 18:1181–1190

Zuzarte M, Gonçalves MJ, Cavaliero C, Canhoto J, Vale-Silva L, Silva MJ, Pinto E, Salgueiro L (2011) Chemical composition and antifungal activity of the essential oils of *Lavandula viridis* L′Her. J Med Microbiol 60(Pt 5):612–618

Part IV
Plant-Derived Products to Protect Fungal Diseases of Plants/Fruits

Chapter 13
Usefulness of Plant Derived Products to Protect Rice Against Fungi in Western Europe

Olívia Matos, Ana Magro and António Mexia

Abstract The ethnopharmacobotanical usages of aromatic plants, from the Mediterranean region, as spices, for human or animal health applications, among other purposes, are well-known. However, natural products from plant origin can also play a role as natural chemical substances for crop and food protection leading to an increased interest on the plants auto protective capacity against pathogenic agents. Search on plant derived bioactive products, which could be less toxic to the environment and more biologically selective than synthetic compounds are of major importance, for rural and agro-industrial economic sectors. Naturally occurring biologically active compounds from plant extracts and essential oils are examples of GRAS compounds, that are of increased importance due to restrictions on pesticides and to environmental and food safety concerns. Rice is a staple food for over half of the world population being one of the cheapest sources of food energy and protein. This crop cereal raises interest in Europe. It is cultivated in the south of Europe for centuries, being Italy the major rice producer and Portugal the main rice consumer. Fungi infection and the inherent occurrence of mycotoxins can be responsible for serious economic losses and public health risks. Knowledge about the origin of the growth of toxigenic fungi is a prerequisite to the

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establishment of mycotoxins control programs. Rice samples collected in Portuguese farms or from factories were analyzed for fungal infection. Several fungi taxa were isolated (*Absidia*, *Alternaria*, *Aspergillus*, *Bipolaris*, *Botrytis*, *Chaetomium*, *Curvularia*, *Cunninghamella*, *Epicoccum*, *Fusarium*, *Geotrichum*, *Helicoma*, *Nigrospora*, *Penicillium*, *Pyricularia*, *Rhizopus*, *Scytalidium*, *Stemphylium*, *Sordaria*, *Trichoconiella*, *Trichoderma*, *Trichothecium*, and *Ulocladium*), some of them being potentially mycotoxigenic. The knowledge on the plant synthesis of substances potentially useful to control microbial infections, led to evaluate the bioactivity of extracts, and essential oils of a set of aromatic plants against fungi affecting rice. We enhance the good results achieved with *Cuminum cyminum, Laurus nobilis, Mentha pulegium, Origanum vulgare*, and *Satureja montana* both with extracts and essential oils against *Aspergillus candidus, Aspergillus flavus*, *Aspergillus niger*, *Botrytis cinerea*, *Cladosporium cucumerinum*, *Fusarium culmorum* and *Penicillium sp*.

Keywords Aromatic plants · Bioactive products · Rice crop · Antifungal potential

13.1 Introduction

The increased public concern over the level of pesticide residues on food encouraged researchers to look at other solutions apart from synthetic pesticides. Naturally occurring bioactive compounds from plants are generally assumed to be more acceptable and less hazardous than synthetic compounds for food and crop protection (Bishop and Reaga 1998). Effectively, plant bioactive products related to auto protective capacity against pathogenic agents, which are registered food grade materials, could be used as alternative antimicrobial treatments.

Aromatic plants and their bioactive constituents can play a role as generally regarded as safe (GRAS) substances for crop and food protection leading to an increased interest on these plants (Mari and Guizzardi 1998; Qin et al. 2004; Ippolito et al. 2005).

The replacement of synthetic fungicides by natural plant products has been referred as a success when applied to control fungi affecting food or stored grains like *Penicillium* sp. (Link). (Chen 1990; Neri et al. 2006), *Botrytis cinerea* (Pers.) (Matos and Barreiro 2004; Matos et al. 2008; Matos 2011a), *Fusarium* sp. (Link) and *Aspergillus* sp. (Fr.: Fr.), (Matos and Ricardo 2006; Magro et al. 2008, 2010), and other phytopathogenic fungi on several crops (Cowan 1999; Matos et al. 1999, 2001).

13.1.1 Rice Cultivation in Western Europe

Rice is a staple food for over half of the world population, being the primary source of human nutrition and one of the cheapest sources of food energy and protein. It is grown on approximately 146 million ha, i.e., more than 10 % of the total available land for agriculture (Cantrell 2002; Mejia 2003).

Rice is one of the most consumed food plants since antiquity, it is impossible to determine precisely when man began to cultivate it. There are two cultivated species of rice, *Oryza sativa* L. and *Oryza glaberrima* Steud, the first coming from the south and southwest Asia (Asian Continent) and the second from the Niger River Delta (African Continent) (Maurici 1999). However, in Europe only the cultivars 'indica,' 'japonica,' and 'aromatic' of *Oryza sativa* are cultivated. Rice was known in Europe only after the expedition of Alexander the Great to India. The Arabs brought it to the Iberian Peninsula at the time of its conquest in 711. In the middle of the fifteenth century was introduced in Italy and France, spreading its cultivation for the rest of the world as a result of European conquests. Portugal played an important role on the introduction of rice in the American Continent. Effectively, in 1694 rice appears in the South Carolina and in the early eighteenth century, it begins to be produced in South America (Vianna e Silva 1969).

In Portugal, the first written references to the cultivation of rice correspond to the reign of King Dinis (1279–1325). In those times, it was only for the tables of rich people. A strong incentive for the production of this cereal was given by King Jose (XVIII century) and wetland, under flooding, especially in the estuaries of major rivers was used for such purpose. However, the adoption of poor cultivation techniques at that time gave rise to areas of "standing water" supporting the development of mosquitoes and other insects, which led to strong opposition from the local people, which attributed responsibility to the crop of various diseases such as malaria. As a consequence rice culture was even prohibited, however, such decision was not enforced in practice (Viana e Silva 1975).

The expansion of rice culture in Portugal took place around 1909, after the drafting of rules for the preparation of land and water management (irrigation and drainage), and a large number of different cultivars were extensively studied for yield improvement through plant breeding programs.

Presently, the major producers of rice in Europe are Italy, Spain, Portugal, Greece, and France (Fernandes 2003). Portugal is the biggest consumer per capita of milled rice in Europe (15 kg per person and per year) and is the third producer with an annual paddy rice production of about 129,000 tonnes of *japonica* variety (short-grain), 26,000 tonnes of *indica* variety (long-grain) distributed mainly by Sado, Tejo, and Mondego river Valleys. Besides national production, 98,000 tonnes of rice are imported to satisfy consumer needs (Brites et al. 2006; INE 2007).

13.1.2 Diseases Affecting Rice

Rice is one of the most important crops for food security, contributing almost a quarter of the calories to mankind (Pennisi 2010), and it is also a staple crop of economic importance in many countries. It is estimated that its production (600 million tons in 2000) will have to increase by 40 % in 2030. This increase will have to face less land and water for rice cultures as well as fewer fertilizers and chemicals. According to the FAO, diseases, insects, and weeds are responsible for yield losses of up to 25 % in rice (Ribot et al. 2008). Diseases are considered major constraints in rice production and are mainly caused by fungi, bacteria, or viruses (Wopereis et al. 2009).

13.1.3 Fungi Diseases of Rice on the Field

There are several diseases affecting rice in the field, likely rice blast, sheat blight, brown spot, leaf scald, narrow brown spot, stem rot, sheath rot, bakanae, and false smut. The three major diseases of rice worldwide are the rice blast, sheath blight, and false smut (Wopereis et al. 2009).

Rice blast disease is one of the most devastating of all cereal diseases worldwide and also the most important in Europe. It is estimated that each year rice blast causes harvest losses of 10–30 % of the global rice yield (Talbot 2003), but even 10 % is significant, being sufficient to feed 60 million people for 1 year. Rice blast disease has been found in more than 85 countries (Kato 2001), leading to serious epidemics throughout rice-growing regions of the world, and contributing significantly to the cost of rice production. Today, blast-preventive, low-cost measures include the burning of crop residues, such as diseased straw and stubble, planting of disease-free seed, avoiding excess nitrogen-based fertilizer, water-seeding, and growth under conditions of continuous flooding. However, these low-impact control measures are rarely efficient enough under blast-favorable conditions. Understanding the biology of rice blast disease is, therefore, of particular significance, because it offers the promise of developing new and durable disease control strategies (Skamnioti and Gurr 2009). This disease is caused by the hemibiotrophic fungal pathogen *Magnaporthe grisea* [(T.T. Hebert) M.E. Barr] (Kou and Wang 2011). This fungus can infect all aerial parts of the rice plant, causing leaf blast, neck and panicle blast, collar rot, and node blast.

Rice sheath blight is another fungal disease caused by the soil-borne necrotrophic pathogen *Rhizoctonia solani* (J.G. Kühn), which has a broad host range and occurs in most rice producing areas (Zou et al. 2000). The disease appears both on sheaths and on laminar portion of leaves, and on most prominent and common symptom caused by *R. solani* is sheath blight. The asexual phase of *R. solani*, sclerotia, can survive in the soil for 2 years and are spread quickly during field preparation and flood application (Webster and Gunnell 1992).

Rice false smut is caused by hemibiotrophic *Ustilaginoidea virens* [(Cooke) Takah]. Although it has long been considered a minor disease (Ou 1972), rice false smut has become a serious problem in recent years in at least some rice-growing areas, such as Asia (Ashizawa et al. 2010). Prior to the heading stage, the fungus invades rice spikelets and the grains of the panicle are covered by powdery, dark green spores during maturation (Ou 1972). False smut may occupy any part of the panicle, which results in yield loss. In addition, *U. virens* produces ustiloxin that is poisonous to humans and other animals (Koiso et al. 1992). Since this disease is visible only after panicle exsertion, the use of fungicides is not effective to prevent yield loss.

Fungicides, resistant cultivars, agronomical practises, and biotechnological methods have been developed and used to control these important diseases (Ribot et al. 2008).

13.1.4 Fungi Diseases of Rice on Storage

Paddy rice is a seasonal crop in Portugal, so storage of paddy and milled rice is of major importance for year-round availability. The storage of cereals is a specific ecosystem, subject to several correlated factors, like temperature, relative humidity, moisture content, oxygen availability, which are difficult to manage.

According to Mew and Gonzales (2002) *Pyricularia grisea* (anamorph) Sacc. (Cooke), the rice blast pathogen, although considered the most important rice pathogen, has the lowest incidence when screening rice seeds for fungi contamination.

Presently, it is not possible to find data in the literature compare the incidence of storage fungi in rice in Portugal and in Europe to the results obtained from Asia and America. To solve this problem, the fungi diversity in rice stored was studied in Portugal. The results obtained from compilation of data from several countries are resumed in the Table 13.1.

In storage, the development of fungi, especially *Aspergillus* spp. and *Penicillium* spp., is an unsolved problem. These fungi are responsible for rice quantitative and qualitative losses and are mycotoxins potential producers. Mycotoxins are hazardous to animal and human health and constitute a factor for economic losses in food products worldwide (Omidbeygi et al. 2007; Pitt and Hocking 2009).

13.2 Mycotoxin Contamination

Mycotoxin production is unavoidable and at times unpredictable, which makes it a unique challenge to food safety. Mycotoxin contamination often occurs in the field prior to harvest. Post-harvest contamination can occur if the drying is delayed and along the storage of the crop if moisture is allowed to exceed critical values for

Table 13.1 Fungi identified from rice samples, worldwide (Manabe and Tsuruta 1991; Pitt et al. 1994; Tonon et al. 1997; Broggi and Moltó 2001; Pacin et al. 2002; Maity et al. 2004; Park et al. 2005; Makun et al. 2007; Pitt and Hocking 2009)

Genus	Species
Absidia	*A. corymbifera*
Acremonium sp.	
Alternaria	*A. alternata*, *A. longissima,* and *A. tenuissima*
Arthrium	*A. phaeospermum*
Aspergillus	*A. aureus, A. avenaceus, A. awamori, A. candidus, A. carbonarius, A. clavatus, A. conicus, A. elegans, A. flavus, A. fumigatus, A. giganteus, A. glaucus, A. japonicus, A. melleus, A. niger, A. ochraceus, A. parasiticus, A. penicillioides, A. phoenicis, A. restrictus, A. sclerotiorum, A. sydowii, A. tamarii, A. terreus, A. ustus, A. versicolor*, and *A.wentii*
Bipolaris	*B. oryzae* and *B. sorghicola*
Chaetomium	*C. brasiliense, C. funicola*, and *C. globosum*
Cladosporium	*C. cladosporioides* and *C. herbarum*
Colletotrichum sp.	
Curvularia	*C. aeria*, *C. affinis, C. eragrostidis, C. geniculatus, C. lunata, C. oryzae,* and *C. pallescens*
Cryptococcus	*C. neoformans*
Diplodia	*D. maydis*
Drechslera	*D. sorokiniana*
Emericella	*E. nidulans*
Epicoccum	*E. nigrum*
Eupenicillium	*E. cinnamopurpureum, E. hirayama,* and *E. ochrosalmoneum*
Eurotium	*E. amstelodami, E. chevalieri, E. herbariorum, E. montevidense, E. pseudoglaucum, E. repens, and E. rubrum*
Fusarium	*F. acuminatum, F. chlamydosporum, F. graminearum, F. heterosporum, F. moniliforme, F. oxysporum, F. proliferatum, F. semitectum, F. solani, and F. verticilloides*
Geosmithia	*Geosmithia sp*
Geotrichum	*G. candidus*
Helminthosporium	*Helminthosporium* sp.
Lasiodiplodia	*L. theobromae*
Macrophomina	*M. phaseolina*
Mucor	*M. hiemalis* and *M. racemosus*
Neosartorya	*N. fischeri*
Nigrospora	*N. oryzae*
Paecilomyces	*P. variotii*
Penicillium	*P. aurantiogriseum, P. capsulatum, P. chrysogenum, P. citreonigrum, P. citrinum, P. corylophilum, P. crustosum, P. cyclopium, P. decumbens, P. diversum, P. expansum, P. funiculosum, P. glabrum, P. granulatum, P. griseofulvum, P. herquei, P. implicatum, P. islandicum, P. janthinellum, P. notatum, P. oxalicum, P. piceum, P. puberulum, P. purpurescens, P. purpurogenum, P. roquefortii, P. roseopurpureum, P. rugulosum, P. thomii, P. variabile, P. verrucosum, P. viridicatum*, and *P. waksmanii*
Phoma	*P. glomerata* and *P. herbarum*
Rhizomucor	*R. pussillus*

(continued)

Table 13.1 (continued)

Genus	Species
Rhizopus	*R. oryzae and R. stolonifer*
Scopulariopsis	*S. brevicaulis*
Sordaria sp.	
Syncephalastrum	*S. racemosum*
Talaromyces	*T. wartmanii*
Themoascus	*T. crustaceus*
Trichoconiella	*T. padwickii*
Trichoderma	*T. viride*
Trichothecium	*T. roseum*
Ulocladium	*U. chartarum*
Wallemia	*W. sebi*

fungi growth. However, mycotoxin contamination is less commonly reported for rice than for many other cereal crops (Tanaka et al. 2007).

The major mycotoxigenic fungi in rice belongs to *Aspergillus* sp. (Reddy et al. 2004), *Fusarium sp*. (Abbas et al. 1999), and *Penicillium* sp. (Makun et al. 2007), and fungal invasion effects can be recognized by the discolorated aspect of glume or grain, loss of quality, and viability and presence of mycotoxins. Reddy et al. (2008) in their study on the incidence of mycotoxigenic fungi and on the relevance of mycotoxins on rice, reported aflatoxins, fumonisins, trichothecenes, ochratoxin A, cyclopiazonic acid, patulin, zearalenone, deoxynivalenol (DON), citrinin, gliotoxin, and sterigmatocystin as the most important.

Aflatoxin B1 (AFB1), fumonisin B1, and ochratoxin A are reported as the most toxic for mammals having hepatotoxic, teratogenic, and mutagenic activity and causing damage such as toxic hepatitis, hemorrhage, edema, immunosuppression, hepatic carcinoma, equine esophageal cancer, and nephrotoxicity (Norred 1993; Altuntas et al. 2003). AFB1 has been classified as a group 1 human carcinogen and fumonisin B1 and B2 as group 2B carcinogens by the International Agency for Research on Cancer (Reddy et al. 2008).

Considering that decontamination of mycotoxins from contaminated food or complete elimination of mycotoxins from food commodities seems difficult or even impossible, prevention is the best control strategy.

13.3 Strategies to Control Fungi

Strategies to reduce the risk associated with fungi incidence on rice are essential and must be improved on a permanent basis.

At present sustainable control strategies, like Integrated Pest Management as part of integrated production schemes, include either indirect control measures such as cultural practises, the adoption of resistant varieties, or direct control measures like chemical control, through synthetic fungicides or antifungal plant

products, with risk periods and spraying times being determined by risk assessment methodologies and action thresholds (economic thresholds) adjusted to each pest problem.

13.3.1 Cultural Practices

Several cultural practices like early sowing, amounts and type of nitrogen fertilizers, appropriate level of phosphates, or even silicium are indicated as cultural measures to reduce disease severity on rice (Lima 1998).

Portuguese rules for Integrated Production impose water and soil analysis on a regular temporal scale and fertilization should be adjusted to the analytical results up to a total nitrogen maximum amount of 180 kg/ha to the "indica" varieties and to 160 kg/ha to the "japonica" varieties, with the use of nitrates being excluded (Costa et al. 2003). Minimum tillage is recommended and only officially registered varieties should be sown, with sowing densities around 180–200 kg/ha, when the average temperature above soil reaches 13 °C, usually in April. Water must be removed from the paddy fields 8–10 days before harvesting, and mechanical harvest must occur with a grain moisture content of 20–22 %. The seeds must be dried to a moisture content below 14 % before storage (Fernandes and Rasquilho 2004).

13.3.2 Resistant Varieties

The adoption of resistant varieties by farmers is by far the most efficient control method for fungi. Indeed, resistant varieties for major rice fungi have been breed since the beginning of the last century.

A significant number of resistance genes are known for the major rice diseases, some being specific of "indica" varieties while others occur in "japonica" varieties, only. Mixtures of different varieties carrying different resistance genes seem to be an interesting strategy to control rice fungi (Lima 1998).

An example of recent achievements is the variety called Macassane, released recently in Mozambique by IRRI, with some effectiveness against rice blast on the leaves and roots (Kerr 2011).

13.3.3 Chemical Control

Bordeaux mixtures, based on copper sulfate, have been used to control rice fungi since the beginning of the last century on a world basis. Since than many fungicides from several chemical groups were applied to prevent rice diseases, like dithiocarbamates, mercurials, benzimidazoles, antibiotics (Kasugamycin), and

triazoles, but for environmental problems or resistance phenomena all have been withdrawn from rice agroecosystems.

Nowadays, apart from copper witch is still in use, strobilurins, namely azoxystrobin are widely used for rice disease control, since 1999. This fungicide inhibits conidia germination and mycelia growth through inhibition of mitochondrial respiration at the cytocrome bc1 pathway. This active ingredient is photodegradable, presents biological activity during 10–12 days, and is absorbed by leaves with a translaminar distribution mainly during the 3 h after spraying. The harvest interval is 28–30 days and the product is highly toxic to algae and aquatic invertebrates and toxic to fish, which cause difficulties to this widespread use in rice agroecosystems. But any present no other active ingredient is allowed under the portuguese Integrated Pest Management rules for rice.

13.4 Use of Plants for Antifungal Purposes

Since pre-historical times plants play a role in man's life, mainly as food and feed products, but being applied in health, as cosmetics and perfumery and in religion. Ancient Greek (Hippocrates, Theophrastus, Dioscroides) and Roman (Pliny, Galen) civilizations were familiarized with medicinal properties of plants (De Pasquale 1984). However, with the discovery of synthetic chemical drugs in the twentieth century, this empirical evidence was rejected and medicine lost confidence in the healing properties of plants (Pepeljnjak et al. 2003).

Plants synthesize a wide variety of secondary metabolites (flavonoids, terpenoids, carotenoids, coumarins, alkaloids, non-protein amino acids, and phenolic compounds). Such compounds are not directly linked to the functions of growth and reproduction of plants, but they result from the adaptive defense mechanisms against biotic and abiotic stresses, and interactions with the environment.

For a long time, plant secondary metabolites have been considered "junk", despite having interesting structures and in many cases, exploitable biological properties. Currently most of these compounds are economically important, and biologically interest and/or therapeutic for humans. They are therefore used as flavors, fragrances, stimulants, dyes, hallucinogens, poisons, or pesticides (Matos 2001; Hill and Wang 2009).

Although better known for its use in herbal medicine, plants have also played a significant role in crop protection and food conservation. Industrial revolution and the organic chemistry development lead to an increased preference for compounds from organic synthesis for the treatment of diseases and pests organisms. However, the excessive and indiscriminate use of organic pesticides has led to the contamination of soil and water, with consequent harm to the health of animals and man (Matos and Ricardo 2006). Alternatives to synthetic pesticides are needed, and substances from plants and microorganisms constitute a viable option. In reality, substances of plant origin can be more advantageous than synthetic

pesticides because they are easily degradable, environmentally low-polluting, and having non-phytotoxic or residual properties.

The plant extracts and essential oils became important again to crop protection after the development of the Integrated Pest Management strategy, which recommends for the control of pests and diseases the minimal use of synthetic pesticides (Magro 2001). Considering that is not possible to stop using substances to control pests and diseases, research into natural pesticides, especially of plant origin, is of major importance.

Present studies on the control of rice fungal contaminants, are in this context. Despite the improvement of hygiene conditions and techniques of food production, food security became increasing by important in a public health perspective. It is estimated that over 30 % of the population in industrialized countries suffers from food poisoning. For instance, in 2000 at least 2 million people died from diseases caused by food poisoning worldwide (WHO 2002).

Storage is a critical milestone on the conservation of food, especially of agricultural products. Changes during this phase, even when not detectable, have an impact throughout the distribution process, processing, and marketing of products (Navarro and Noyes 2002). The effects of the deterioration caused by fungi can lead to total loss of food or health problems, due to the presence of mycotoxins, with serious toxicity risks to consumers (Vargas et al. 2001; Matos 2011b).

Natural products of plant origin have an increasingly importance, because there is still need for research of active natural substances to the protection of agricultural products, particularly to control fungal pathogens of cereals and dried foods stored.

13.5 Aromatic Plants Assayed

Literature revision on antimicrobial usefulness and the results previously achieved (Matos and Ricardo 2006; Matos et al. 2001; Gata-Gonçalves et al. 2003; Magro 2009; Magro et al. 2010; Serrano et al. 2011; Ramos et al. 2011; Teixeira et al. 2012) allows the selection for the present work of the aromatic plants *Cuminum cyminum*, *Laurus nobilis*, *Mentha pulegium*, *Origanum vulgare*, *Satureja montana*, traditionally used as spices or for medicinal purposes.

C. cyminum L. is a native spice from east India and east Mediterranean of the *Apeaceae* family. Seeds of *C. cyminum* are consumed as condiment across the globe, having a distinctive popular aroma, due to their content in the compound cuminaldehyde (Viuda-Martos et al. 2007). *C. cyminum* can also be a source of generally recognized as safe (GRAS) constituents that can be used for fresh food application to increase clean sanitation by reducing to a minimum the microbial population (Gachkar et al. 2007) and by reducing oxidative undesired processes.

The chemical composition of cumin seeds essential oil can present a significant variability due to several factors such as climate, soil composition, vegetative cycle stage, harvest-time, storage conditions, extraction method (El-Sawi and Mohamed

2002; Gachkar et al. 2007; Hajlaoui et al. 2010; Karbin et al. 2009; Lee et al. 2007; Viuda-Martos et al. 2007). Such chemical differences can affect the biological properties of cumin, what reinforce the importance of the cumin integrated analysis of chemical profile and biological pattern. However, the GC–MS analysis showed that 73.0 % of the total compounds of cumin oil were composed by 2-methyl-3-phenyl-propanal (46.6 %) and 1-methyl-3-(1-methylethyl)-benzene (26.4 %). The most representative monoterpene hydrocarbons are 2-β-pinene (3.8 %), γ-terpinene (2.0 %) and 1-isopropylidene-3-n-butyl-2-cyclobutene (3.4 %). Eucalyptol (0.2), trans-pinocarveol (0.2 %), 2,6-dimethyl-3,5,7-octatriene-2-ol (9.5 %), and carvacrol (0.7 %) are the most representative oxygenated monoterpenes, while very low concentrations of oxygenated sesquiterpenes ($\sim$0.1 %.) and sesquiterpene hydrocarbons ($\sim$0.1 %) were detected in the cumin seed oil.

L. nobilis L. belongs to the laurisilva forests that originally covered much of the Mediterranean Basin when the climate of the region was more humid. Laurel forest flora still persist in the mountains of southern Turkey, northern Syria, southern Spain, north-central Portugal, and northern Morocco. *L. nobilis* are used almost exclusively as flavor agents for food preparation, and its leaves have a long shelf life of about 1 year, under normal temperature and humidity. *L. nobilis* leaves are used as a valuable spice and flavoring agent in the culinary and food industry. This plant does not have important uses in traditional medicine but recently has been the subject of scientific research (Conforti et al. 2006). However, Laurel's antiseptic, antibacterial, and antifungal properties are known. Laurel leaves contains about 1.3 % essential oils, consisting of eucalyptol (27.2 %), α-terpinyl acetate (10.2 %), linalool (8.4 %), methyleugenol (5.4 %), sabinene (4.0 %), and carvacrol (3.2 %) (Conforti et al. 2006; Vaughan and Geissler 2009; Ramos et al. 2011).

M. pulegium L. is a plant that occurs spontaneously in the Mediterranean region, in Europe, Asia Minor and northern Iran (Beghidja et al. 2007). It is native from the southwestern Iberian Peninsula being frequent and abundant in Portugal. Antimicrobial activity have been reposted (Sivropoulou et al. 1995; Teixeira et al. 2012) including antifungal effects against soilborne phytopathogenic fungi (Mueller-Riebau et al. 1995). Major components of *M. pulegium* oil are piperitone (38.0 %), piperitenone (33.0 %), alpha-terpineol (4.7 %), and pulegone (2.3 %) (Mahboubi and Haghi 2008; Teixeira et al. 2012).

O. vulgare L. is a well-known culinary aromatic herb, from Lamiaceae family, from Asia, Europe, and northern Africa. It is highly appreciated in gastronomy being also used in folk medicine in the treatment of respiratory disorders, dyspepsia, painful menstruation, rheumatoid arthritis, scrofulosis and urinary tract disorders, expectorant, diaphoretic, and herb (Gruenwald et al. 2000). Studies on the isolation of *O. vulgare* essential oils are known, from different regions of the world including Greece (Daferera et al. 2002), Lithuania (Mockute et al. 2001), India (Bisht et al. 2009), Poland (Figiel et al. 2010), and Italy (Russo et al. 1998), among others are known. Such studies are mainly focused on the chemical composition, but antioxidant and antimicrobial properties were also explored (Faleiro et al. 2005).

The essential oil of *O. vulgare* is mainly composed by oxygenated monoterpenes (40.6 %) and monoterpene hydrocarbons (26.4 %). Within oxygenated monoterpenes, carvacrol (14.5 %), β-fenchyl alcohol (12.8 %), and δ-terpineol (7.5 %) were the major compounds detected, while γ-terpinene (11.6 %) and α-terpinene (3.7 %) were the most abundant monoterpene hydrocarbon detected (Teixeira et al. 2012). Additionally, thymol (12.6 %) and 1-methyl-3-(1-methyl-ethyl)-benzene (6.8 %) represent substantial fractions of *O. vulgar*e essential oil. Carvacrol, thymol, γ-terpinene, and linalool are known to possess strong antioxidant and antifungal properties (Adam et al. 1998; Yanishlieva et al. 1999; Ruberto and Baratta 2000; Aligiannis et al. 2001).

S. montana L. whose center of distribution is located in the eastern part of the Mediterranean area, is an annual or perennial semi-bushy that inhabit arid, sunny, stony, and rocky regions. In Portugal, *S. montana* is spontaneous in the Northeast region and in Madeira islands (Cunha et al. 2007). The genus *Satureja* is known to have several biological properties, such as bactericidal, fungicidal (Azaz et al. 2005; Bezbradica et al. 2005; Cavar et al. 2008), and anti-HIV-1 (Yamasaki et al. 1998; Cavar et al. 2008).

Major constituents of the essential oil are phenols, like carvacrol (30.6 %), thymol (14.1 %), and carvacrol methyl ether (6.3 %). (Serrano et al. 2011). Various flavonoids, flavonoid glycosides (eriodictyol 7-0-rutinoside), phenolic acids (caffeic, ρ-hydroxylbenzoic, ρ-coumaric, ferulic, rosmarinic), tannins (3–8 %), and triterpenes (β-sitosterol-β-D-glucoside, oleanolic acid, ursolic acid, crataegolic acid) have been mentioned as constituents of *S. montana* extracts (Escudero et al. 1985).

13.6 Experimentation

A set of assays was performed either as a first screening for the incidence of fungi on rice cultivated in Portugal, or to evaluate the antifungal potential of a set of aromatic plants from the Mediterranean Region against mycotoxigenic fungi isolated from sampled rice.

13.6.1 Screening for Fungi Incidence on Rice

Rice samples, namely paddy rice, brown rice, and milled rice were collected regularly during the experimental period in a farmer's storage and in a rice mill in different points of the milling process. Five samples per place were collected in sterilized containers and taken into the laboratory.

Rice samples were then divided in sub-samples of 110 grains. The surface of these sub-samples was disinfected with 1 % sodium hypochlorite, for two minutes, as described by Magro et al. (2011) and Pitt and Hocking (2009). Ten dried grains

were placed in Petri dishes with 20 mL of potato dextrose agar (PDA) medium with chloramphenicol (1 %). For each sample, ten replicates were made.

The grains in Petri dishes were incubated at 28 °C for 8 days and then examined under a light stereomicroscope for fungal growth. Isolation of the colonies was made to obtain pure cultures. Slides of fungal growth were prepared and observed under a compound microscope for fungal morphology study.

The identification was carried out using identification keys (Ellis 1971; Carmichael et al. 1980; Domsch et al. 1980; Onions et al. 1981; International Mycological Institute 1991; Hanlin 1997; Malloch 1997; Barnett and Hunter 1998; Samson et al. 2004 and Pitt and Hocking 2009).

13.6.2 Aromatic Plants Analyzed for Antifungal Properties

13.6.2.1 Plant Material

The aromatic plant species *C. cyminum*, *L. nobilis*, *M. pulegium*, *O. vulgare*, *S. montana*, were selected to be assayed against mycotoxigenic fungi. Plant specimens were collected from the field, identified, and voucher specimens were deposited at the Herbarium of INIAV.

13.6.2.2 Preparation of Plant Extracts and Essential Oils

Ethanol and water extracts were obtained by maceration of dried material (150 g) in an Erlenmeyer flask with 1,000 mL ethanol (purity grade 990 g L^{-1}) or with 1,000 mL deionized boiling water using a continuous hot extraction method in a Soxhlet apparatus (30 min, about 100 °C). Plant material was pressed and the resultant liquids were filtered under vacuum through a Buckner funnel (90 mm diameter) with filter paper Whatman n° 1 (Whatman, Maidstone, UK).

The essential oils were obtained by hydro-distillation using a modified Clevenger apparatus in a ratio of 100 g of dry plant material to 700 mL of deionized water. The essential oils were obtained after 3 h of distillation, dried over anhydrous sodium sulfate, filtrated and stored at 4 °C until further analysis. The ethanol extract was poured into a round-bottomed flask and brought to dryness in a rotary evaporator (40 °C; and at about 178 mbar). Finally, plant extracts were freeze dried and stored at −80 °C. The ethanol and the water extracts were redissolved in ethanol or water, respectively, prior the use in individual assays while the essential oils were directly applied.

13.6.2.3 Preparation of Fungus Inocula

The mycotoxigenic fungi *Aspergillus candidus* Link, *Aspergillus flavus* Link, *Aspergillus A. niger* Tiegh.nom.cons., *Botrytis cinerea*, *Fusarium culmorum* (WG Smith) Sacc., *Penicillium islandicum* Sopp obtained from samples of rice grains, were selected as targets to perform the in vitro and in vivo biological evaluations of the antifungal potential of plant extracts and essential oils.

The fungi were grown on PDA media in the dark at 28 °C, for 5–10 days, depending on the fungus, and then used to provide the inocula necessary for the antifungal tests. Petri dishes (9 cm Ø) containing 20 ml of solidified PDA medium were used for all the different antifungal assays.

13.6.3 Assay Procedures

13.6.3.1 Plant Screening for Antifungal Potential

In the first plant, screening for antifungal activity aqueous suspension cultures was prepared by disintegrating the mycelium of each fungus previously grown in PDA media. These fungi suspensions, prepared in 200 ml of an adequate liquid media (Matos and Ricardo 2006), were filtered through a Buckner funnel with nylon nets (pore size 100 μm), under vacuum, in order to ensure their homogeneity, adding liquid media until a spore count of about 5×10^4 per ml was achieved. A liquid inoculum (1 ml) of each of the fungi was pipetted and homogeneously dispersed over the surface of the agar. A 7 mm in diameter hole was pierced in the agar in the center of the dish, to receive 250 μl of the ethanol extract, 500 μl of the aqueous extracts, or 50 μl of the essential oil. Only one plant extract or plant oil was tested per dish, and four replicates of each assayed modality were done. The plates were incubated at 28 °C, for 8 days.

13.6.3.2 Antifungal Tests with Essential Oils Under Saturated Atmospheres in Vitro and in Vivo

To evaluate the potential of the plants essential oils, a method based on "saturated atmospheres" was used, where the samples analyzed and each fungus tested are confined to a particular sealed shell, in this case Petri dishes.

For such in vitro tests, Petri dish containing 20 ml of PDA (sterilized by autoclaving at 121 °C for 15 min) were prepared and inoculated with a disk of mycelium, 0.5 cm in diameter, taken from the periphery of each fungus colony. After this, filter papers (Whatman No. 1, 90 mm Ø) impregnated with 50, 100, 250, 500, or 750 μl of the essential oils were placed in the upper side of the Petri dishes, and the system was sealed with parafilm, and placed in an incubation

chamber at 28 °C for 25 weeks. Replicates without the essential oil were also prepared. The assays were monitorized for fungal growth weekly.

In vivo tests under saturated atmospheres were also performed using the methodology described above with the proviso that a sample of 1 g of sterile rice was placed over the PDA before the application of each plant oil. In these assays doses of 500 and 1,000 μL of essential oils were tested. Four replicates of each assayed modality were prepared.

13.7 Results and Discussion

13.7.1 Screening for Fungi Incidence on Rice

The incidence of field and storage fungi on rice collected from farmers and industry storages with different origins was studied. The totality of isolated and

Table 13.2 Fungi identified in all samples of rice

Taxa	
Genus	Species
Absidia	*A. corymbifera*
Alternaria	*A. alternata, A. arborescens, A. infectoria,* and *A. tenuissima*
Aspergillus	*A. candidus, A. flavus, A. fumigatus, A. niger, A. ochraceus, A. penicillioides, A. sydowi, A. terreus, A. versicolor*, and *A. wentii,*
Bipolaris	*B. australiensis, B. cynodontis, B. hawaiiensis* and *B. oryzae*
Botrytis	*Botrytis* sp.
Chaetomium	*C. globosum, C. spirale,* and *C. trilaterale*
Cunninghamella	*Cunninghamella* sp.
Curvularia	*C. aeria, C. intermedia,, C. lunata*, and *C. pallescens*
Epicoccum	*E. purpurascens*
Eurotium	*E. chevalieri* and *E. rubrum*
Fusarium	*F. equiseti, F. moniliforme (group)*, and *F. semitectum*
Geotrichum	*Geotrichum* sp.
Mucor	*M. racemosus*
Nigrospora	*N. oryzae*
Penicillium	*P. citreonigrum, P. citrinum, P. islandicum, P. rugulosum, P. steckii, and P. viridicatum*
Pyricularia	*P. oryzae*
Rhyzopus	*R. oryzae*
Scytalidium	*Scytalidium* sp.
Sordaria	*S. fimicola*
Stemphylium	*S. botryosum*
Syncephalastrum	*S. racemosum*
Trichoconiella	*T. padwickii*
Trichoderma	*T. harzianum*
Trichothecium	*T. roseum*
Ulocladium	*U. atrum*

Table 13.3 *Taxa* isolated from national and imported rice samples

Taxa	National rice			Imported rice	
	Paddy	Brown	Milled	Brown	Milled
Absidia corymbifera	x	–	–	–	–
Alternaria spp.	x	x	–	–	–
A. alternata	x	x	–	x	x
A. arborescens	x	–	–	–	–
A. infectoria	–	x	–	–	–
A. tenuissima	–	x	–	–	–
Aspergillus sp	–	x	x	x	x
A. candidus	x	x	x	x	x
A. flavus	x	x	–	x	x
A. fumigatus	x	x	x	x	x
A. niger	x	x	–	x	–
A. ochraceus	–	x	–	–	–
A. penicillioides	–	–	x	–	–
A. sydowii	–	x	–	–	–
A. terreus	x	x	–	–	–
A. versicolor	–	x	–	–	–
A. wentii	–	–	–	x	–
Bipolaris spp.	x	x	–	–	–
B. australiensis	x	–	–	–	–
B. cynodontis	–	x	–	–	–
B. hawaiiensis	x	–	–	–	–
B. oryzae	x	x	–	x	–
Botrytis sp.	x	–	–	–	–
Chaetomium globosum	x	–	–	–	–
C. spirale	–	x	–	–	–
C. trilaterale	x	x	–	–	–
Cunninghamella sp.	x	–	–	–	–
Curvularia aeria	x	–	–	–	–
C. intermedia	x	–	–	–	–
C. lunata	x	–	–	–	–
C. pallescens	–	–	–	x	–
Epicoccum purpurascens	–	x	–	–	–
Eurotium chevalieri	–	x	x	x	x
E. rubrum	–	–	–	x	–
Fusarium spp.	–	x	–	x	–
F. equiseti	–	x	–	–	–
F. moniliforme	x	–	–	–	–
F. equiseti	–	x	–	–	–
F. moniliforme	x	–	–	–	–
Geotrichum sp.	x	x	–	–	–
Helicoma sp.	–	–	–	x	–
Nigrospora oryzae	x	x	x	–	–
Mucor racemosus	x	–	–	–	–
Penicillium spp.	x	x	x	x	–

(continued)

Table 13.3 (continued)

Taxa	National rice			Imported rice	
	Paddy	Brown	Milled	Brown	Milled
P. citreonigrum	–	–	–	x	–
P. citrinum	x	–	–	x	–
P. islandicum	x	x	–	x	–
P. rugulosum	–	x	–	–	–
P. steckii	–	x	x	–	–
P. viridicatum	x	–	–	–	–
Pyricularia oryzae	–	x	–	–	–
Rhizopus oryzae	x	–	–	x	–
Scytalidium sp.	x	x	–	–	–
Sordaria fimicola	x	–	–	–	–
Stemphylium botryosum	–	x	–	–	x
Syncephalastrum racemosum	x	x	–	–	–
Trichoconiella padwickii	x	x	x	x	x
Trichoderma harzianum	x	x	–	–	–
Trichothecium roseum	x	x	–	–	–
Ulocladium atrum	–	x	–	–	–
Total of identified Taxa	37	38	9	21	8

identified taxa is present in Table 13.2. Table 13.3 presents the different fungi isolated from national and imported rice.

The results obtained clearly show that in Portugal, the national rice showed greater fungi diversity then the imported rice. In fact, in present study a total of 73 taxa were identified, 68 taxa in the national rice, and 24 in that imported rice. The fungi with higher incidence either in national or in imported were *Alternaria* spp., *Aspergillus candidus*, *A. flavus*, *A. niger*, *A. terreus Thom*, *Fusarium* spp, *Nigrospora* sp. Zimm., *Penicillium* spp., *Rhyzopus* sp. Ehrenb., *Trichoconiella padwickii* (Ganguly) B.L.Jain, and *Trichoderma* sp. Pers.. The higher incidence of storage fungi agrees with reports of other authors that over time field fungi tend to decrease, being replaced by storage fungi (Mourato 1984; Manabe and Tsuruta 1991; Pitt and Hocking 2009).

Considering the economic and nutritional importance of rice, it is desirable to improve and monitorize rice production both in field and under storage conditions to reduce fungi incidence. Fungus contamination of rice is a problem to be solved due the fact that some of the isolated fungi are potential mycotoxin producers, such is the case of *Aspergillus* sp. and *Penicillium* sp.. However, the presence of a particular fungus does not necessarily imply the existence of dangerous mycotoxins and the diet should be as diversified as possible in order to minimize the impact of toxins.

13.7.2 Plant Screening for Antifungal Potential

The antifungal potential of aqueous (250 μl), ethanol (250 μl) extracts, and of the essential oil (50 μl) of *C. cyminum*, *L. nobilis*, *M. pulegium*, *O. vulgare*, and *S. montana* were preliminarily tested in vitro against *A. candidus, A. flavus, A. niger, B. cinerea, F. culmorum*, and *P. expansum*. Results observed, 8 days after incubation (Table 13.4), showed antifungal efficacy for all the plants and formulations used, despite the low concentrations used.

As a whole the *Aspergillus* species were less affected by all the extracts while *F. culmorum* and *P. islandicum* were the more susceptible. Aqueous extracts were the less effective reaching a maximum of 60.8 ± 2.5 %, observed for *O. vulgare* tested on *F. culmorum*. The extracts of *O. vulgare* obtained in ethanol were also the most active against all the fungi reaching 67.3 ± 2.8 % against *F. culmorum.* The essential oils were the most active plant material against this set of fungi. The oils of *O. vulgare* (73.5 ± 4.6 %), *S. montana* (66.2 ± 4.2), and *C. cyminum* (64.2 ± 10.4) were the most active also against *F. culmorum*. The results observed above conduced to the selection of essential oils to be used in the assays under saturated atmosphere.

13.7.3 Saturated Atmosphere In Vitro

The effects of the different doses of *L. nobilis* and *M. pulegium* essential oil assayed in saturated atmosphere in vitro on the growth of *A. candidus, A. niger, F. culmorum, and P. islandicum* are presented in Tables 13.5 and 13.6.

The growth of *F. culmorum* was completely inhibited by *L. nobilis* essential oil along the 25 weeks when the doses of 100, 250, and 500 μL were used, while the total inhibition of *P. islandicum* was achieved only with the doses of 250 and 500 μL. The dose of 50 μL was inefficient for these two fungi while with 100 μL *P. islandicum* was controlled only for the three first weeks. *L. nobilis* oil was efficient to control the growth of *A. candidus* and *A. niger* only when the dose of 750 μl was applied, along 16 and 19 weeks, respectively, with the dose of 500 μL for 4 and 7 weeks, respectively, and with the dose of 250 μL for 1 and 3 weeks, respectively.

As shown in Table 13.6 all the doses of *M. pulegium* essential oil were efficient to control the growth of *F. culmorum* and *P. islandicum* along the 25 weeks of the assays. In the case of *A. candidus* and *A. niger* such level of inhibition was observed only for the dose of 500 μL. With the dose of 250 μL the total growth inhibition was achieved only during 14 and 12 weeks, respectively against *A. candidus* and *A. niger*, while the dose of 100 μL was efficient for both fungi only during the first 6 weeks.

Table 13.4 Effects of ethanol, aqueous and essential oil of *Cuminum cyminum, Laurus nobilis*, *Mentha pulegium, Origanum vulgare*, *Satureja montana* on the growth of *Aspergillus flavus, Aspergillus niger, Aspergillus candidus, Botrytis cinerea, Fusarium culmorum, and Penicillium islandicum* isolated from rice samples. Results, observed 8 days after incubation at 28 °C, expressed as percentage of the control

Aromatic Plants	Extract	Dose (μL)	*A. flavus*	*A. niger*	*A. candidus*	*B. cinerea*	*F. culmorum*	*P. islandicum*
C. cyminum	Oil	50	60.0 ± 9.9	55,4 ± 5,3	n.t.	42.9 ± 5.8	64.2 ± 10.4	n.t.
	Ethanol	250	55.8 ± 7.1	54.6 ± 4.8	n.t.	38.3 ± 5.9	56.8 ± 9.6	n.t.
	Aqueous	500	44.6 ± 6.9	34.7 ± 5.9	n.t.	29.0 ± 3.1	47.7 ± 2.5	n.t.
L. nobilis	Oil	50	49.8 ± 2.9	28.7 ± 4.2	45.6 ± 7.5	n.t.	45.7 ± 3.3	52.3 ± 4.9
	Ethanol	250	37.3 ± 8.6	27.9 ± 6,2	43.3 ± 6.4	n.t.	48.2 ± 5.2	51.6 ± 5.3
	Aqueous	500	27.4 ± 3.0	26.4 ± 3.9	39.6 ± 5.7	n.t.	28.5 ± 4.0	49.7 ± 5.6
M. pulegium	Oil	50	46.8 ± 7.1	48.6 ± 5.1	51,3 ± 5.6	43.7 ± 5.2	51.5 ± 4.6	66.3 ± 5.8
	Ethanol	250	40.2 ± 6.7	45.9 ± 6.3	49.3 ± 6.2	42.9 ± 4.8	43.2 ± 5.2	63.5 ± 6.1
	Aqueous	500	38.9 ± 6.4	43.8 ± 5.3	46.7 ± 5.8	39.9 ± 4.6	33.3 ± 4.2	57.4 ± 5.3
O. vulgare	Oil	50	55.9 ± 4.8	58.5 ± 5.9	n.t.	55.8 ± 5.5	73.5 ± 4.6	n.t.
	Ethanol	250	58.4 ± 2.9	55.7 ± 4.3	n.t.	54.9 ± 5.9	67.3 ± 2.8	n.t.
	Aqueous	500	53.1 ± 3.8	51.9 ± 4.9	n.t.	52.4 ± 6.0	60.8 ± 2.5	n.t.
S. montana	Oil	50	57.4 ± 5.1	52,6 ± 5.3	n.t.	n.t.	66,2 ± 4,2	n.t.
	Ethanol	250	59.7 ± 4.6	56.2 ± 4.8	n.t.	n.t.	64,5 ± 3,4	n.t.
	Aqueous	500	14,3 ± 1,7	14.3 ± 1.6	n.t.	n.t.	15,3 ± 1.7	n.t.

Table 13.5 Effects of essential oil of *Laurus nobilis* on the growth of *Fusarium culmorum*, *Penicillium islandicum*, *Aspergillus candidus*, and *Aspergillus niger* tested in a saturated atmosphere in vitro during 25 weeks at the temperature of 28 °C

Fungi	Volume (μL)	Incubation time (weeks)																								
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Fusarium culmorum	500	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	250	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	100	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	50	○	○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	Control	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Penicillium islandicum	500	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	250	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	100	○	○	○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	50	○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	Control	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Aspergillus candidus	750	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	●	●	●	●	●
	500	○	○	○	○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	250	○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	Control	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Aspergillus niger	750	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	●	●
	500	○	○	○	○	○	○	○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	250	○	○	○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	Control	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●

Table 13.6 Effects of essential oil of *Mentha pulegium* on the growth of *Fusarium culmorum*, *Penicillium islandicum*, *Aspergillus candidus,* and *Aspergillus niger* tested in a saturated atmosphere in vitro during 25 weeks at the temperature of 28 °C

Fungi	Volume (μL)	Incubation time (weeks)																								
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Fusarium culmorum	500	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	250	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	100	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	Control	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Penicillium islandicum	500	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	250	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	100	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	Control	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Aspergillus candidus	500	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	250	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	●	●	●	●	●	●	●
	100	○	○	○	○	○	○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	Control	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Aspergillus niger	500	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	250	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	●	●	●	●	●	●	●	●	●
	100	○	○	○	○	○	○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	Control	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●

Table 13.7 Effects of essential oil of *Laurus nobilis* on the growth of *Fusarium culmorum*, *Penicillium islandicum*, *Aspergillus candidus,* and *Aspergillus niger* tested in a saturated atmosphere in vivo during 25 weeks at the temperature of 28 °C

Fungi	Volume (μL)	Incubation time (weeks)																								
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Fusarium culmorum	1,000	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	Control	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Penicillium islandicum	1,000	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	Control	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Aspergillus candidus	1,000	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	●	●
	Control	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Aspergillus niger	1,000	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	Control	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●

Table 13.8 Effects of essential oil of *Mentha pulegium* on the growth of *Fusarium culmorum*, *Penicillium islandicum*, *Aspergillus candidus*, and *Aspergillus niger* tested in a saturated atmosphere in vivo during 25 weeks at the temperature of 28 °C

Fungi	Volume (μL)	Incubation time (weeks)																								
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Fusarium culmorum	500	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	Control	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Penicillium islandicum	500	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	Control	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Aspergillus candidus	500	○	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	●	●	●	●	●	●	●	●	●
	Control	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Aspergillus niger	500	○	○	○	○	○	○	○	○	○	○	○	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	Control	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●

13.7.4 Saturated Atmosphere In Vivo

The antifungal effects of the essential oils of *L. nobilis* and *M. pulegium*, assayed under saturated atmosphere in vivo, are presented in the Tables 13.7 and 13.8.

In these assays, the doses of 1,000 μL, for the case of *L. nobilis*, and 500 μL, for *M. pulegium*, were selected based on the results obtained from the previous assays performed in saturated atmospheres in vitro. The growth of *F. culmorum*, *P. islandicum* and *A. niger* was completely inhibited along the 25 weeks with the application of 1,000 μL of *L. nobilis* oil, while for *A. candidus* these level of control was observed only during 20 weeks.

The total fungi inhibition was observed with *M. pulegium* along the 25 weeks in the cases of *F. culmorum* and *P. islandicum* while this plant oil was efficient for *A. candidus* and *A. niger* only during 12 and 11 weeks, respectively.

The previous results allow the conclusion that the set of plants used showed considerable good activity against the mycotoxigenic fungi used as biological targets.

The assays performed to screen aromatic plants for their antifungal potential, revealed that the selected plants species are good sources of bioactive products to control all the mycotoxigenic fungi used as biological targets. This in vitro test allows to the selection of the plant species and the plant extract to be used in the following tests for long periods of observation. However, it is necessary to increase the dose of essential oil in the case of in vivo assays for total growth inhibition of all studied fungi. Indeed, Omidbeygi et al. (2007) and Matan et al. (2006) claim that when the test is done in vivo, the effectiveness of the oils is decreased and it is necessary to increase the dose to achieve the same level of effectiveness.

From the five plants screened for antifungal potential *L. nobilis* and *M. pulegium* were selected to perform the assays under saturated atmospheres based on their less palatability impact in food. In fact best results were observed with *C. cyminum, O. vulgare,* and *S. montana*. However, under saturated atmospheres tests only the oil formulations were applied and an increased amount of the doses was needed.

Analyzing the results obtained with *L. nobilis*, they are in accordance with others authors (Suhr and Nielsen 2003; Angelini et al. 2006) for studies with fungi responsible for food spoilage. However, when Suhr and Nielsen (2003) and Angelini et al. (2006) compared the effectiveness of *L. nobilis* essential oil with other essential oils such as *Syzygium aromaticum* (L.) Merrill and Perry, *Cinnamomum zeylanicum* J.Presl and *Thymus* sp. L., they found that *L. nobilis* essential oils were the most effective. Also Panizzi et al. (1995) compared the antifungal properties of *Laurus* essential oils to these from *Cedrus atlantica* (Man), assayed in solid media, on *Fusarium moniliforme* var. *subglutinosus, Pyricularia grisea, A. niger* and *B. cinerea* and found better results with *L. nobilis* then with *C. atlantica*.

Otherwise, the tests performed by Atanda et al. (2007), in liquid medium with the essential oil of *L. nobilis* reduced the concentration of aflatoxins, although it stimulated the growth of *A. parasiticus* Speare but when the essential oil was placed in contact with sorghum grain, it was found that this reduced the growth of *A. parasiticus*. It is thought that this reduction is not related only to the volatility of the active compounds in oil, but is also from possible effects resulting from direct contact with the grain.

According to Bouchra et al. (2003) *M. pulegium* essential oil has a moderate activity when compared to the *Origanum compactum* Benth. and *Thymus glandulosus* Lag. ex H. del Villar essential oils. However, when comparing the antifungal action of *M. pulegium* to *Lavandula dentata* L., *Salvia aegyptiaca* L., *Calamintha officinalis* Moench, and *Rosmarinus officinalis* L., those authors referred the high efficacy of *M. pulegium* essential oil against *B. cinerea*. These differences in behavior are related to the essential oils composition. In fact, the major constituent of the *M. pulegium* essential oil are pulegone and piperitona, while those of *Origanum compactum* and *Thymus glandulosus* are carvacrol and thymol, respectively, which are described in the literature as compounds with high fungicidal activity (Chami et al. 2005; Valero et al. 2006; Michiels et al. 2007). However, Cardenas-Ortega et al. (2005) demonstrated that piperitone in low concentrations completely inhibited the growth of *Aspergillus flavus*.

13.8 Conclusions

Despite the wide knowledge of aromatic plants and their bioactive components for different uses it is important to understand their biological mode of action for new applications in human health, agriculture, and the environment. Plant extracts and essential oil components can constitute effective alternatives or complements to synthetic compounds without secondary effects.

The selection of a method based on the concentration of essential oil vapor, the so-called saturated atmospheres, to protect cereals revels to be advantageous due to the smaller influence on the final flavor of the cereal product. Residual problems were also avoided because oils are not in direct contact with the grains. Besides the use of plant oils do not change the food flavor since the oil component volatized easily through aeration.

Comparative analysis of the two methodologies used for in vitro assays confirmed the importance of methodology selection. For instance *L. nobilis* and *M. pulegium* oils showed very different performances when tested in vitro using the common method or the saturated atmospheres.

The use of plant products to treat or to prevent the incidence of fungi during storage increases importance due to the high losses of rice due to storage fungus contamination. Effectively losses caused by fungi reaching 25–30 % are registered every year mainly in developing countries, such as the Portuguese speaking countries like Mozambique and Guiné-Bissau. Plant products are cheaper sources

of bioactive products, and in general farmers and rural communities have good practical knowledge on their utility and use. The present work can supply information on methods that can be easily applied to different crop storage systems.

References

Abbas HK, Cartwright RD, Xie W, Mirocha CJ, Richard JL, Dvorak TJ, Sciumbato GL, Shier WT (1999) Mycotoxin production by *Fusarium proliferatum* isolates from rice with *Fusarium* sheath rot disease. Mycopathologia 147:97–104

Adam K, Sivropoulou A, Kokkini S, Lanaras T, Arsenakis M (1998) Antifungal activities of *Origanum vulgare* subsp. *hirtum*, *Mentha spicata*, *Lavandula angustifolia*, and *Salvia fruticosa* essential oils against human pathogenic fungi. J Agric Food Chem 46:1739–1745

Aligiannis N, Kalpoutzakis E, Mitaku S, Chinou IB (2001) Composition and antimicrobial activity of the essential oils of two *Origanum* species. J Agric Food Chem 49:4168–4170

Altuntas I, Delibas N, Doguc DK, Ozmen S, Gultekin F (2003) Role of reactive oxygen species in organophosphate insecticide phosalone toxicity in erythrocytes *in vitro*. Toxicol *In Vitro* 17:153–157

Angelini P, Pagiotti R, Menghini A, Vianello B (2006) Antimicrobial activities of various essential oils against foodborne pathogenic or spoilage moulds. Ann Microbiol 56:65–69

Ashizawa T, Takahashi M, Moriwaki J, Hirayae K (2010) Quantification of the rice false smut pathogen *Ustilaginoidea virens* from soil in Japan using real-time PCR. Eur J Plant Pathol 128:221–232

Atanda OO, Akpan I, Oluwafemi F (2007) The potential of some spice essential oils in the control of *A. parasiticus* CFR 223 and aflatoxin production. Food Control 18:601–607

Azaz AD, Kurkcuoglu M, Satil F, Baser HC, Tumen G (2005) *In vitro* antimicrobial activity and chemical composition of some *Satureja* essential oils. Flavour Frag J 20:87–591

Barnett HL, Hunter BB (1998) Illustrated genera of imperfect Fungi. APS Press, Minnesota

Beghidja N, Bouslimani N, Benayache F, Benayache S, Chalchat JC (2007) Composition of the oils from *Mentha pulegium* grown in different areas of the East of Algeria. Chem Nat Compd 43:481–483

Bezbradica DI, Tomovic JM, Vukasinovic MS (2005) Composition and antimicrobial activity of essential oil of *Satureja montana* L. collected in Serbia and Montenegro. J Essent Oil Res 17:462–465

Bishop CD, Reagan J (1998) Control of the storage pathogen *Botrytis cinerea* on Dutch white cabbage (*Brassica oleracea* var. *capitata*) by the essential oil of *Melaleuca alternifolia*. J Essent Oil Res 10:57–60

Bisht D, Chanotiya CS, Rana M, Semwal M (2009) Variability in essential oil and bioactive chiral monoterpenoid compositions of Indian oregano (*Origanum vulgare* L.) populations from northwestern Himalaya and their chemotaxonomy. Ind Crops Prod 30:422–426

Bouchra C, Achouri M, Hassani LMI, Hmamouchi M (2003) Chemical composition and antifungal activity of essential oils of seven Moroccan Labiatae against *Botrytis cinerea* Pers:Fr. J Ethnopharmacol 89:165–169

Brites CM, Guerreiro M, Modesto ML (2006) Arroz Carolino: uma jóia da nossa gastronomia. COTArroz, Salvaterra de Magos

Broggi LE, Moltó GA (2001) Fungi associated with rice at Entre Rios province, Argentina. Toxigenic capacity of *Fusarium graminearum* and *Microdochium nivale* isolates. Mycotox Res 17:96–107

Cantrell RP (2002) Arroz: porquê é tão essencial para a segurança e estabilidade global. Perspectivas Económicas, publicação electrónica do Departamento de Estado—USA. In:

Pereira D, Bandeira D, Quincozes E (eds) Cultivo do arroz irrigado no Brasil. Sistemas de Produção, Embrapa, Brasil
Cárdenas-Ortega NC, Zavala-Sánches MA, Aguirre-Rivera JR, Pérez-Gonzalez C, Pérez-Gutiérrez S (2005) Chemical composition and antifungal activity of essential oil of *Chrysactinia mexicana* gray. J Agric Food Chem 3:4347–4349
Carmichael JW, Kendrick WB, Conners IL, Singler L (1980) Genera of hyphomycetes. Univ Alberta Press, Manitoba
Cavar S, Maksimovic M, Solic M, Jerkovic-Mujkic A, Besta R (2008) Chemical composition and antioxidant and antimicrobial activity of two *Satureja* essential oils. Food Chem 111:648–653
Chami N, Bennis S, Chami F, Aboussekhra A, Remmal A (2005) Study of anticandidal activity of carvacrol and eugenol in vitro and *in vivo*. Oral Microbiol Immunol 20:106–111
Chen MD (1990) Study on effects of citrus antifungal preservative papers and films on citrus storage. In: Proceedings of international citrus symposium, Guangzhou, China, 5–8:814–815
Conforti F, Statti G, Uzunov D, Menichini F (2006) Comparative chemical composition and Antioxidant activities of wild and cultivated *Laurus nobilis* L. Leaves and *Foeniculum vulgare* subsp. *piperitum* (Ucria) Coutinho seeds. Biol Pharm Bull 29:2056–2064
Costa ASV, Calouro MFS, Cavaco M (2003) Produção integrada das culturas de arroz, milho e cereais de Outono/Inverno—fertilização. MADRP/Direcção-Geral da Protecção das Culturas, Oeiras
Cowan MM (1999) Plant products as antimicrobial agents. Clin Microbiol Rev 12:564–582
Cunha AP, Ribeiro JA, Roque OR (2007) Plantas aromáticas em Portugal caracterização e utilizações. Fundação Calouste Gulbenkian, Lisboa
Daferera DJ, Tarantilis PA, Polissiou MG (2002) Characterization of essential oils from Lamiaceae species by Fourier transform Raman spectroscopy. J Agric Food Chem 50:5503–5507
De Pasquale A (1984) Pharmacognosy: the oldest modern science. J Ethnopharmacol 11:1–16
Domsch KH, Gams W, Anderson TH (1980) Compendium of soil fungi. Academic Press, London
El-Sawi Salma A, Mohamed MA (2002) Cumin herb as a new source of essential oils and its response to foliar spray with some micro-elements. Food Chem 77:75–80
Ellis MB (1971) Dematiaceous hyphomycetes. Commonwealth Mycological Institute, England
Escudero J, López JC, Rabanal RM, Valverde S (1985) Secondary metabolites from *Satureja* Species. New Triterpenoid from *Satureja acinos*. J Nat Prod 48:128–131
Faleiro L, Miguel G, Gomes S, Costa L, Venancio F, Teixeira A, Figueiredo AC, Barroso JG, Pedro LG (2005) Antibacterial and antioxidant activities of essential oils isolated from *Thymbra capitata* L. (Cav.) and *Origanum vulgare* L. J Agric Food Chem 53:8162–8168
Fernandes APS (2003) Utilização de Lemna minor na Avaliação de Efeitos Tóxicos de Herbicidas em Ecossistemas Orizícolas. Relatório de Trabalho de Fim de Curso de Engenharia Agronómica, Instituto Superior de Agronomia, Universidade Técnica de Lisboa, Lisboa
Fernandes I, Rasquilho A (2004) Protecção e Produção integradas das culturas de arroz, milho e cereais de Outono/Inverno—práticas culturais. MADRP/Direcção-Geral da Protecção das Culturas, Oeiras
Figiel A, Szumny A, Gutiérrez-Ortíz A, Carbonell-Barrachina ÁA (2010) Composition of oregano essential oil (*Origanum vulgare*) as affected by drying method. J Food Eng 98:240–247
Gachkar L, Yadegaria D, Rezaeib MB, Taghizadehc M, Astanehc SA, Rasooll (2007) Chemical and biological characteristics of *Cuminum cyminum* and *Rosmarinus officinalis* essential oils. Food Chem 102:898–904
Gata-Gonçalves L, Nogueira JMF, Matos O, Sousa RB (2003) Photoactive extract from *Thevetia peruviana* with antifungal properties against *Cladosporium cucumerinum*. J Photoch Photob B: Biol 70:51–54
Gruenwald J, Brendler T, Jaenicke C (2000) PDR for herbal medicines. Medical Economic Company, Montvale

Hajlaoui H, Mighri H, Noumi E, Snoussi M, Trabelsi N, Ksouri R, Bakhrouf A (2010) Chemical composition and biological activities of Tunisian *Cumin cymin* L. essential oil: a high effectiveness against *Vibrio* spp. strains. Food Chem Toxicol 48:2186–2192

Hanlin RT (1997) Ilustrated genera of ascomycetes. APS Press, Minnessota

Hill L, Wang TL (2009) Approaches to the analysis of plant-derived natural products. In: Osbourn AE, Lanzotti V (eds) Plant-derived natural products. Springer, New York

INE (Instituto Nacional de Estatística) (2007) Portugal Agrícola 1980–2006. Instituto Nacional de Estatística, Lisboa

International Mycological Institute (1991) Mycological techniques. CAB International, London

Ippolito A, Schena L, Pentimone I, Nigro F (2005) Control of postharvest rots of sweet cherries by pre- and postharvest applications of *Aureobasidium pullulans* in combination with calcium chloride or sodium bicarbonate. Postharvest Biol Technol 36:245–252

Karbin S, Rad AB, Arouiee H, Jafarnia S (2009) Antifungal activities of the essential oils on postharvest disease agent *Aspergillus flavus*. Adv Environ Biol 3:219–225

Kato H (2001) Rice blast disease. Pesticide Outlook 12.1:23–25. In: Skamnioti P, Gurr SJ (2009) Against the grain: safeguarding rice from rice blast disease. Trends Biotechnol 27:141–150

Kerr G (2011) Facts about rice fungus. http://www.livestrong.com/article/545483-facts-about-rice-fungus. Accessed Dec 2011

Koiso Y, Natori M, Iwasaki S, Sato S, Sonoda R, Fujita Y, Yaegashi H, Sato Z (1992) Ustiloxin: a phytotoxin and a mycotoxin from false smut balls on rice panicles. Tetrahedron Lett 33:4157–4160

Kou Y, Wang S (2011) Toward an understanding of the molecular basis of quantitative disease resistance in rice. J Biotechnol 20:283–290

Lee KW, Kim YJ, Lee HJ, Lee CY (2007) Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. J Agric Food Chem 51:7292–7295

Lima A (1998) A Piriculariose e o estudo da variabilidade genética de uma população portuguesa de Pyricularia grisea Sacc. de arroz. Tese de Doutoramento em Engenharia Agronómica. Universidade Técnica Lisboa—Instituto Superior Agronomia, Lisboa

Magro A (2001) A problemática dos fungos no armazenamento de produtos agrícolas duráveis. Trabalho de síntese para provas de acesso a Assistente de Investigação. Instituto de Investigação Científica Tropical (IICT), Lisboa

Magro A (2009) Avaliação das actividades fungistáticas e fungicidas de extractos de plantas e seus constituintes em produtos agrícolas e secos armazenados. Tese de Doutoramento em Biologia. Universidade Técnica Lisboa—Instituto Superior Agronomia, Lisboa

Magro A, Faro A, Barata M, Matos O, Bastos M, Carolino M, Lima A, Mexia A (2008) Rastreio da incidência de fungos em arroz para consumo. In: Proceedings of I Encontro Nacional de Produção Integrada e VIII Encontro Nacional de Protecção Integrada Escola Superior Agrária de Ponte de Lima

Magro A, Matos O, Bastos M, Carolino M,Mexia A (2010) The use of essential oils to protect rice from storage fungi In: Carvalho OM, Fields PG, Adler CS, Arthur FH, Athanassiou CG, Campbell JF, Fleurat-Lessard F, Flinn PW, Hodges RJ, Isikber AA, Navarro S, Noyes RT, Riudavets J, Sinha KK, Thorpe GR, Timlick BH, Trematerra P, White NDG (eds). Proceedings 10th international working conference stored products protection, Lisboa

Magro A, Pera S, Barata M, Carolino M, Bastos M, Matos O, Mexia A (2011) The incidence of fungi in stored rice. Integr Prot Stored Prod IOBC/wprs Bulletin 69:19–24

Mahboubi M, Haghi G (2008) Antimicrobial activity and chemical composition of *Mentha pullegium* L. essential oil. J Ethnopharmacol 119:325–327

Maity JP, Chakraborty A, Saha A, Santra SC, Chanda S (2004) Radiation-induced effects on some common storage edible seeds in India infested with surface microflora. Rad Phys Chem 71:1065–1072

Makun HA, Gbodi TA, Akanya OH, Salako EA, Ogbadu GH (2007) Fungi and some mycotoxins contaminating rice (*Oryza sativa*) in Niger State, Nigeria. African J Biotechnol 6:99–108

Malloch D (1997) Moulds—isolation, cultivation, identification. http://www.botany.utoronto.ca/. Accessed Sep 2005

Manabe M, Tsuruta O (1991) Mycoflora and mycotoxins in stores rice grain. In: Chelkowski J (ed) Cereal grain mycotoxins, fungi and quality in drying and storage. Elsevier, New York
Mari M, Guizzardi M (1998) The post-harvested phase: emerging technologies for the control of fungal diseases. Phytoparasitica 26:59–66
Matan N, Rimkeeree H, Mawson AJ, Chompreeda P, Haruthaithanasan V, Parker M (2006) Antimicrobial activity of cinnamom and clove oils under modified atmosphere conditions. Int J Food Microbiol 107:180–185
Matos OC (2001) Estudo de Espécies Nativas Portuguesas. Produção de Substâncias com Actividade Biológica (Study of Portuguese native plant species. Production of substances with biological activity). Tese das Provas de Acesso à Categoria de Investigador Auxiliar. Instituto Nacional Investigação Agrária-Estação Agronómica Nacional, Oeiras
Matos OC (2011a) As plantas como fonte de produtos naturais biologicamente ativos (Plants as source of natural biologically active products). Agrorrural. Contributos Científicos (INRB/Imprensa Nacional-Casa da Moeda eds)
Matos OC (2011b) Controlo de qualidade de produtos agrícolas e de bens de consumo e serviços no ramo alimentar. Agrorrural. Contributos Científicos (INRB/Imprensa Nacional-Casa da Moeda eds)
Matos OC, Barreiro MG (2004) Safety use of bioactive products of plant origin for the control of post harvested fungal diseases of "Rocha" pear. Proceedings of IV Simposium Ibérico de Maturação e pós-colheita: Frutos Hortícolas, Lisboa
Matos OC, Ricardo CP (2006) Screening of plants against fungi affecting crops and stored foods. In: Carpinella MC (ed) Rai MK. Naturally occurring bioactive compounds, Elsevier BV
Matos OC, Baeta JM, Silva MJ, Ricardo CPP (1999) Sensibility of patogenic fungi to *Chelidonium majus* L. extracts. J Ethnopharmacol 66:151–158
Matos OC, Gata-Gonçalves L, Pais I, Lima JC, Baião F, Pinto Ricardo C (2001) Light activated antifungal and antibacterial effects of plant extracts and plant cell culture media. Melhoramento 37:288–292
Matos OC, Santos M, Barreiro MG (2008) Use of a new natural plant compound against post-harvest 'Rocha' pear rots. In: Almudi RO, Falcón JV,Mairal AF (eds) Avances en maduración y post-recolección de frutas y hortalizas, Editorial ACRIBIA, S. A., Zaragoza, Espanha
Maurici JA (1999) El arroz. Principales enfermedades, plagas e malas hierbas. BASF
Mejia D (2003) Post-harvest operations compendium. AGSI/FAO, Rome. http://www.fao.org/inpho/content/compend/text/CH22_01.htm. Accessed July 2009
Mew TW, Gonzales P (2002) A hand book of rice seed born fungi. IRRI; Science Publishers, Inc. http://hdl.handle.net/123456789/1016. Accessed Dec 2011
Michiels J, Missotten J, Fremaut D, De Smet S, Dierick N (2007) In vitro dose–response of carvacrol, thymol, eugenol and trans-cinnamaldehyde and interaction of combinations for the antimicrobial activity against the pig gut flora. Livestock Sci 109:157–160
Mockute D, Bernotiene G, Judzentiene A (2001) The essential oil of *Origanum vulgare* L. ssp. *vulgare* growing wild in Vilnius district (Lithuania). Phytochemistry 57:5–69
Mourato MA (1984) Alguns aspectos do problema dos fungos em produtos armazenados. Revista Ciências Agrárias pp 49–65
Mueller-Riebau F, Berger B, Yegen O (1995) Chemical composition and fungitoxic properties to phytopathogenic fungi of essential oils of selected aromatic plants growing wild in Turkey. J Agric Food Chem 43:2262–2266
Navarro S, Noyes R (2002) the mechanics and physics of modern grain aeration management. CRC Press, Boca Raton
Neri F, Mari M, Brigati S (2006) Control of *Penicillium expansum* by plant volatile compounds. Plant Pathol 55:100–105
Norred WP (1993) Fumonisins-mycotoxins produced by *Fusarium moniliforme*. J Toxicol Environ Hlth 38:309–328
Omidbeygi M, Barzegar M, Hamidi Z, Naghdibadi H (2007) Antifungal activity of thyme, summer savory and clove essential oils against *Aspergillus flavus* in liquid medium and tomato paste. Food Control 18:1518–1523

Onions AHS, Allsopp D, Eggins HOW (1981) Smith's introduction to industrial mycology. Edward Arnold Publishers Ltd, London

Ou SH (1972) Rice disease. Commonwealth Mycological Institute, Kew

Pacin AM, González HHL, Etcheverry M, Resnik SL, Vivas L, Espin S (2002) Fungi associated with food and feed commodities from Ecuador. Mycopathologia 156:87–92

Panizzi L, Flamini G, Cioni PL, Perruci S, Morelli I (1995) Propreit antimicotiche in vitro di alcuni oliessenziali su ife e spore di muffe contaminanti gli alimenti. *Acta II Congresso Nazionale di Chimica degli Alimenti*. In: Flamini G, Cioni PL (eds). Activity of plant extracts, essential oils and pure compounds against fungi contaminating foodstuff and causing infections in Human beings and Animals: a six-year experience (1995–2000)

Park JW, Choi SY, Hwang HJ, Kim YB (2005) Fungal mycoflora and mycotoxins in Korean polished rice destined for humans. Int J Food Microbiol 103:305–314

Pennisi E (2010) Armed and dangerous. Science (New York, NY) 327:804. In: Galhano R, Talbot NJ (2011) The biology of blast: understanding how *Magnaporthe oryzae* invades rice plants. Fungal Biol Rev 25:61–67

Pepeljnjak S, Kosalec I, Kalodera Z, Kustrak D (2003) Natural antimycotics from Croatian plants. In: Rai M, Mares D (eds) Plant-derived antimycotics. Current trends and future prospects. Food Products Press, New York

Pitt JI, Hocking AD (2009) Fungi and food spoilage. Springer, New York

Pitt JI, Hocking AD, Bhudhasamai K, Miscamble BF, Wheeler KA, Tanboon-Ek P (1994) The normal mycoflora of commodities from Thailand. 2. Beans, rice, small grains and other commodities. Int J Food Microbiol 23:35–53

Qin G, Tian S, Xu Y (2004) Biocontrol of postharvest diseases on sweet cherries by four antagonistic yeast in different storage conditions. Postharvest Biol Technol 31:51–58

Ramos C, Teixeira B, Batista I, Matos O, Serrano C, Neng NR, Nogueira JMF, Nunes ML. Marques A (2011) Antioxidant and antibacterial activity of essential oil and extracts of bay laurel Laurus nobilis Linnaeus (Lauraceae) from Portugal. Natural Product Research, 0:1–12

Reddy CS, Reddy KRN, Kumar RN, Laha GS, Muralidharan K (2004) Exploration of aflatoxin contamination and its management in rice. J Mycol Pl Pathol 34:816–820

Reddy KRN, Reddy CS, Abbas HK, Abel CA, Muralidharan K (2008) Mycotoxigenic Fungi, Mycotoxins, and management of rice grains. Toxin Rev 27:287–317

Ribot C, Hirsch J, Balzergue S, Tharreau D, Nottéghem J, Lebrun M, Morel J (2008) Susceptibility of rice to the blast fungus, *Magnaporthe grisea*. J Plant Physiol 165:114–124

Ruberto G, Baratta MT (2000) Antioxidant activity of selected essential oil components in two lipid model systems. Food Chem 69:167–174

Russo M, Galletti GC, Bocchini P, Carnacini A (1998) Essential oil chemical composition of wild populations of Italian oregano spice (*Origanum vulgare* ssp. *hirtum* (Link) Ietswaart): a preliminary evaluation of their use in chemotaxonomy by cluster analysis. 1. Inflorescences. J Agric Food Chem 46:3741–3746

Samson RA, Hockstra E, Frisvad JC (2004) Introduction to Food and airborne fungi, 7th edn. CBC.Centraalbureaus voor Schimmelcultures Utrecht

Serrano C, Matos O, Teixeira B, Ramos C, Neng N, Nogueira J, Nunes ML, Marques A (2011) Antioxidant and antimicrobial activity of *Satureja montana* L. extracts. J Sci Food Agric 91:1554–1560

Skamnioti P, Gurr SJ (2009) Against the grain: safeguarding rice from rice blast disease. Trends Biotechnol 27:141–150

Sivropoulou A, Kokkini S, Lanaras T, Arsenakis M (1995) Antimicrobial activity of mint essential oils. J Agric Food Chem 43:2384–2388

Suhr KI, Nielsen PV (2003) Antifungal activity of essential oils evaluated by two different application techniques against rye bread spoilage fungi. J Appl Microbiol 94:665–674

Talbot NJ (2003). On the trail of a cereal killer: exploring the biology of Magnaporthe grisea. Annual Review of Microbiology. In: Galhano R, Talbot NJ (2011) The biology of blast: Understanding how *Magnaporthe oryzae* invades rice plants. Fungal Biol Rev 25:61–67

Tanaka K, Sago Y, Zheng Y, Nakagawa H, Kushiro M (2007) Mycotoxins in rice. Int J Food Microbiol 119:59–66

Teixeira B, Marques A, Ramos C, Batista I, Serrano C, Matos O, Neng NR, Nogueira JMF, Saraiva JA, Nunes ML (2012) European pennyroyal (*Mentha pulegium*) from Portugal: chemical composition of essential oil and antioxidant and antimicrobial properties of extracts and essential oil. Ind Crops Prod 36:81–87

Tonon SA, Marucci RS, Jerke G, García A (1997) Mycoflora of paddy and milled rice produced in the region of Northeastern Argentina and Southern Paraguay. Int J Food Microbiol 37:231–235

Valero D, Valverde JM, Martínez-Romero D, Guillén F, Castillo S, Serrano M (2006) The combination of modified atmosphere packaging with eugenol or thymol to maintain quality, safety and functional properties of table grapes. Postharvest Biol Technol 41:317–327

Vargas EA, Preis RA, Castro L, Silva CMG (2001) Co-ocurrence of aflatoxins B1, B2, G1, G2, zearalenone and fumisonin B1 in Brasilian corn. Food Addit Contam 18:981–986

Vaughan JG, Geissler C (2009) The new Oxford book of food plants. Oxford University Press, Oxford

Vianna e Silva M (1969) Arroz. Fundação Calouste Gulbenkian, Lisboa

Viana e Silva M (1975) A cultura do arroz. Editora Clássica, Lisboa

Viuda-Martos M, Ruíz-Navajas Y, Fernández-López J, Pérez-Álvarez JA (2007) Chemical composition of the essential oils obtained from some spices widely used in Mediterranean region. Acta Chim Slov 54:921–926

Webster RK, Gunnell PS (1992) Compendium of rice diseases. American Phytopathological Society, St. Paul

WHO (World Health Organization) (2002) Food safety and foodborne illness. In: Burt S (2004) Essential oils: their antibacterial properties and potential applications in food—a review. Int J Food Microbiol 94:223–253

Wopereis MCS, Defoer T, Idionoba P, Diack, S, Dugué MJ (2009) Reference 24 major diseases in rice. PLAR–IRM curriculum: technical manual 105–107

Yamasaki K, Nakano M, Kawahata T, Mori H, Otake T, Ueba N (1998) Anti-HIV-1 activity of herbs in Labiatae. Biol Pharm Bull 2:829–833

Yanishlieva NV, Marinova EM, Gordon MH, Raneva VG (1999) Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. Food Chem 64:59–66

Zou JH, Pan XB, Chen ZX, Xu JY, Lu JF, Zhai WX, Zhu LH (2000) Mapping quantitative trait loci controlling sheath blight resistance in two rice cultivars (*Oryza sativa* L.). Theor Appl Genet 101:569–573

Chapter 14
Plant Bioactive Metabolites for Cereal Protection Against Fungal Pathogens

Caterina Morcia, Giorgio Tumino and Valeria Terzi

Abstract The yield, the quality, and the nutritional safety of cereals can be seriously affected by the development, both in the field and during storage, of fungi that can produce several classes of mycotoxins, characterized by strong negative effects on the human and animal's health. For the diseases control, modern strategies include the development of cereal resistant varieties obtained through classical or innovative breeding strategies, together with the application of biological agents and new "green" chemicals as pesticide. To this purpose, Medicinal and Aromatic Plants are a source of natural products useful in "environmental-friendly" organic and conventional farming. This chapter reviews the wide range of essential oils (EOs) and natural compounds that have been demonstrated to have fungicide and fungistatic effects against mycotoxigenic fungi of cereals. It also addresses their mechanisms of action against fungi and their impact on plants tissues both at cellular and molecular levels.

14.1 Introduction

The growing environmental impact of human activities makes it urgent to address the problem of sustainability. Also in agriculture the requirement for innovative sustainable solutions will be one of the topics of this century. Modern agriculture

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will have to be able to increase productivity per hectare, reducing losses due to abiotic and biotic agents. For the diseases control, modern strategies include the use of marker-assisted selection breeding, genetic engineering of resistance genes, biological agents, and new "green" chemicals as pesticide.

In the course of evolution the plants have developed many chemical compounds of defense, both constitutive and broad-spectrum, and induced following the recognition of a specific pathogen (Bennett and Wallsgrove 1994). Plants are therefore a potential source of new natural products usable in "environmental-friendly" organic and conventional farming. Aromatic and medicinal plants (AMPs) have a long history of use in all Mediterranean areas for food preservation and for pest control (Ait-Ouazzou et al. 2011a, b; Lang and Buchbauer 2011).

In particular several plant species belonging to *Myrtaceae*, *Lauraceae*, *Rutaceae*, *Lamiaceae*, *Asteraceae*, *Cupressaceae* are "aromatics" and synthesize and secrete aroma/essential oils (EOs) characterized by two or three major components at quite high concentrations (up to 80 %) compared with other components present only in trace amounts. The main group is composed of terpenes and terpenoids, and the other group contains aromatic and aliphatic constituents. The monoterpenes are the most representative molecules, constituting 90 % of EOs and comprising a great variety of structures. These secondary metabolites can be overproduced in plants under specific environmental conditions, in all or in specific organs and structures. Many of them are the results of an adaptive evolutionary process in which convergent, independent, and repeated evolution steps are brought to the development, from ancestor genes, of structurally definite secondary metabolites in different plant groups, reflecting the plasticity of responses to environment and the different adaptive strategies of species. The variability of EOs composition is therefore related both to the plant genomes and to the geographical origin of the plants. Mediterranean areas are undoubtedly a unique reservoir of genetic resources of both wild and cultivated AMPs, that can have stronger and innovative applications as antimicrobials in several fields related to crop yield, food and feed production, safety, and quality. In a recent review (Morcia et al. 2011a) we have reported several studies focused on the use of EOs and their components for the control of phytopathogenic fungi that can affect the quality and safety of crops. In this short review, results more focused on fungi that can affect specifically cereals during the growing period or during the grain storage step are reported. The attention on cereals is justified by their fundamental role in human and animal nutrition at planetary level (Fig.14.1) and by the fact that fungal growth, occurring during the crop development in the field or during grains storage period, causes significant quantitative and qualitative losses, with lowering of the nutritional properties and accumulation of highly dangerous mycotoxins (Cardenas-Ortega et al. 2005; Paster et al. 1995).

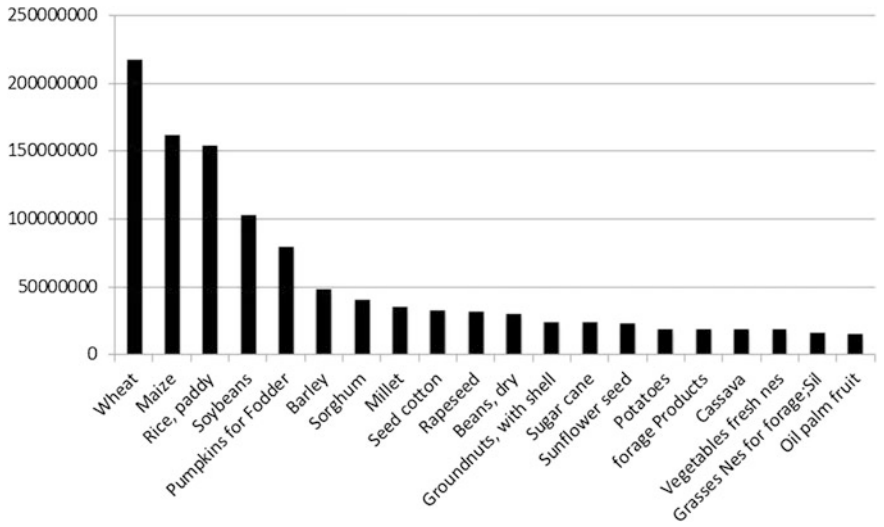

Fig. 14.1 The 20 most important crops in 2010. In the graph are reported the total areas (Ha) harvested in the world. Cereals, like wheat, maize, rice, barley, sorghum and millet, are within the first eight (according to FAOSTAT data, publicly available at http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor)

14.2 EOs and Natural Compounds Against Fungi that Attack Cereals Mainly in the Fields

Some plant-derived products are already marketed as fungicides: GC-3™, 30 % cottonseed oil, 30 % corn oil, 23 % garlic extract, labeled for powdery mildew on various crops; Organocide™, 5 % sesame oil, labeled broadly for several fungal diseases and insects; Promax™, 3.5 % thyme (*Thymus vulgaris*) oil, labeled for several soil-borne fungal diseases and nematodes on many crops; Trilogy™, 70 % clarified hydrophobic extract of neem (*Azadirachta indica*) oil, labeled for several diseases and insects; Sporatec AG™, 18 % rosemary oil, 10 % clove oil, and 10 % thyme oil, labeled for several bacterial and fungal diseases on many crops; Milsana™, 5 % giant knotweed (*Reynoutria sachalinensis*) extract.

Starting from the results obtained with these commercial compounds as environmental-friendly alternatives to synthetic ones, several studies have been now activated to evaluate the efficacy of wide range of EOs to protect cereals against field and postharvest pathogens. Table 14.1 summarizes successful in vitro or in vivo results obtained using EOs or their components for the control of fungi that can affect the cereal growth in open field.

In the work of Morcia et al. (2012) the antifungal activities of terpinen-4-ol, eugenol, carvone, 1,8-cineole (eucalyptol), and thymol, taking part in the composition of EOs, were observed in vitro on *Fusarium subglutinans*, *Fusarium cerealis*, *Fusarium verticillioides*, *Fusarium proliferatum*, *Fusarium oxysporum*, *Fusarium*

Table 14.1 EOs and EO compounds tested for their fungistatic/fungicidal effects against cereal pathogenic fungi

EO/compound	Effective against	Experimental conditions	Effects	References
Thymol	*Fusarium subglutinans*, *F. cerealis*, *F. verticillioides*, *F. proliferatum*, *F. oxysporum*, *F. sporotrichioides*, *Aspergillus tubingensis*, *A. carbonarius*, *Alternaria alternata*, *Penicillium*	In vitro	EC90 values ranged from 0.032 to 0.022	Morcia et al. (2011b)
Eugenol	*Fusarium subglutinans*, *F. cerealis*, *F. verticillioides*, *F. proliferatum*, *F. oxysporum*, *F. sporotrichioides*, *Aspergillus tubingensis*, *A. carbonarius*, *Alternaria alternata*, *Penicillium*.	In vitro	EC90 values ranged from 0.048 to 0.033	Morcia et al. (2011b)
Carvone	*Fusarium subglutinans*, *F. cerealis*, *F. verticillioides*, *F. proliferatum*, *F. oxysporum*, *F. sporotrichioides*, *Aspergillus tubingensis*, *A. carbonarius*, *Alternaria alternata*, *Penicillium*	In vitro	EC90 values ranged from 0.21 to 0.039	Morcia et al. (2011b)
1,8-Cineole	*Fusarium subglutinans*, *F. cerealis*, *F. verticillioides*, *F. proliferatum*, *F. oxysporum*, *F. sporotrichioides*, *Aspergillus tubingensis*, *A. carbonarius*, *Alternaria alternata*, *Penicillium*	In vitro	EC90 values ranged from 1.3 to 0.25	Morcia et al. (2011b)
Terpinen-4-ol	*Fusarium subglutinans*, *F. cerealis*, *F. verticillioides*, *F. proliferatum*, *F. oxysporum*, *F. sporotrichioides*, *Aspergillus tubingensis*, *A. carbonarius*, *Alternaria alternata*, *Penicillium*	In vitro	EC90 values ranged from 0.2 to 0.08	Morcia et al. (2011b)
Methyleugenol	*Alternaria humicola*, *Rhizoctonia solani* and *Fusarium solani*	In vitro	IC_{50} 0.052 μg ml^{-1}	Dan et al. (2010)
Ageratum conyzoides EO	*Aspergillus flavus*	In vitro	IC_{50} 0.1 μg ml^{-1} and complete inhibition of aflatoxin production	Nogueira et al. (2010)

(continued)

Table 14.1 (continued)

EO/compound	Effective against	Experimental conditions	Effects	References
Lantana indica EO	*Aspergillus flavus*	In vitro	1.5 μg ml^{-1} (growth) 0.75 μg ml^{-1} (aflatoxin) completely inhibited	Kumar et al. (2010)
Cestrum nocturnum	*Botrytis cinerea*, *Colletotrichum capsici*, *Fusarium oxysporum*, *Fusarium solani*, *Phytophthora capsici*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*	In vitro	MIC values ranged from 62 (*P. capsici*) to 500 μg ml^{-1}	Al-Reza et al. (2010)
Metasequoia glyptostroboides	*Fusarium oxysporum*, *Fusarium solani*	In vitro	MIC values of 62 and 125 μg ml^{-1} respectively	Bajpai and Kang (2010)
Spiraea alpina EO	*Rhizoctonia solani* and *Fusarium graminearum*	In vitro	Growth inhibition of 95.1 % for 125 μg ml^{-1}	Teng et al. (2010)
Agapanthus africanus EO	*Puccinia triticina*	In vivo (wheat)	Reduction of leaf rust pustules by 40 %	Cawood et al. (2010)
Zingiber officinale, *Curcuma longa*, *Reynoutria sachalinensis* EOs	*Blumeria graminis*	In vivo (wheat)	70 – 75 % of CPLAD reduction	Vechet et al. (2009)
Melaleuca alternifolia EO	*Blumeria graminis hordei*	In vivo (barley)	Complete reduction of powdery mildew symptoms	Morcia et al. (2011a)

sporotrichioides, *Aspergillus tubingensis*, *Aspergillus carbonarius*, *Alternaria alternata*, *Penicillium*. All the compounds showed toxic effects on in vitro mycelium growth of all the fungal strains, although with different level of potency.

The EO of *Asarum heterotropoides* F. Schmidt var. *mandshuricum* exhibited antimycotic properties against several phytopathogens, including *Alternaria* spp., *Fusarium* spp., *Ustilago maydis* (Liu et al. 2007). The main component of the oil is methyleugenol (59 %), that in vitro inhibits the colony growth of plant pathogens *Alternaria humicola*, *Colletotrichum gloeosporioides*, *Rhizoctonia solani*, *Phytophthora cactorum,* and *Fusarium solani* (IC_{50} value of 0.052 μg ml^{-1}) by microorganellar membrane disruption (Dan et al. 2010).

The mycelial growth and aflatoxin B_1 production by *Aspergillus flavus* are inhibited by the EO of *Ageratum conyzoides* (Asteraceae), in yeast extract-sucrose broth assay (Nogueira et al. 2010). At crude oil concentrations ≥0.1 μg ml^{-1} the aflatoxin production is completely inhibited and the fungal growth is reduced 50 % or more when compared with control. The GC/MS analysis indicated that the EO major components were precocene II (46.35 %), precocene I (42.78 %), cumarine (5.01 %), and trans-caryophyllene (3.02 %) (Nogueira et al. 2010).

Also the leaf EO of *Lantana indica* exhibited in vitro antifungal and antiaflatoxigenic properties against *A. flavus* (Kumar et al. 2010). The fungitoxic activity of the *L.indica* EO, tested by disk diffusion assay (for fungal growth) and by SMKY broth-medium culture (for spectrophotometric determination of aflatoxin B_1), completely inhibited fungal growth and aflatoxin production at 1.5 and 0.75 μg ml^{-1}, respectively (Kumar et al. 2010).

The antimicrobial activity of the EO and various organic extract from flowers of *Cestrum nocturnum* (Al-Reza et al. 2010) and leaf of *Metasequoia glyptostroboides* (Bajpai and Kang 2010) was investigated. In vitro mycelial growth of *Botrytis cinerea*, *Colletotrichum capsici*, *F. oxysporum*, *F. solani*, *Phytophthora capsici*, *R. solani,* and *Sclerotinia sclerotiorum* was tested on PDA medium by disk diffusion assay method. The EO and the methanol extract of *C. nocturnum* exhibited antifungal activity against all tested fungi, with MIC values ranged from 62 (*P. capsici*) to 500 μg ml^{-1}. Moreover, EO activity resulted in 80–100 % spore germination inhibition at 500 μg ml^{-1} concentration (Al-Reza et al. 2010). The disk diffusion of *F. oxysporum* and *F.solani* was markedly affected by *M.glyptostroboides* EO, with MIC values of 62 and 125 μg ml^{-1}, respectively (Bajpai and Kang 2010).

The EO of *Spiraea alpina*, an aromatic plant growing in the Southwest China, had strong antifungal activity against *R. solani* and *Fusarium graminearum*, with mycelial growth inhibition of 95.1 % for 125 μg ml^{-1}. Linolenic acid ethyl ester (18.3 %), palmitic acid (9.84 %), cinnamic acid (5.92 %) and its derivatives, trans-phytol (5.38), ethyl palmitate (5.21), and linalool (2.79) were the main components of the oil, as showed by gas chromatography/mass spectrometry analysis (Teng et al. 2010).

The crude leaf extract of *Agapanthus africanus* was shown to promote in vitro activity of the PR-proteins β-1,3-glucanase, chitinase, and peroxidase in susceptible (Thatcher) and resistant (Thatcher/Lr15) near-isogenic wheat lines, both for plants uninfected and infected with the obligate biotrophic pathogen *Puccinia*

triticina. In susceptible Thatcher line the *A. africanus* extract treatment resulted in the reduction of leaf rust pustule by 40 %, in comparison with control. Moreover, the germ tube development of *P.triticina* spores was significantly inhibited (3 % germination) (Cawood et al. 2010).

Particularly effective seems to be the EOs action against *Blumeria graminis*, as demonstrated in vivo both in wheat and in barley. More in details, plant extracts from ginger (*Zingiber officinale* Roscoe), curcuma (*Curcuma longa* L.) rhizomes, and giant knotweed (*R. sachalinensis* L.) leaves were strongly efficient to control *B. graminis* in wheat under field conditions (Vechet et al. 2009). Pre-infection treatment by the plant extracts on naturally infected susceptible wheat cultivar Kanzler reduced the cumulative proportion of leaf area disease (CPLAD) by 70–75 %, in comparison with untreated control. CPLAD reduction for the resistant cultivars Alka, Ramiro and Vasta ranged from 80 to 95 %. Moreover the progress of powdery mildew was monitored over 50 days after treatment, on both treated and untreated Kanzler cultivar. For the treated plants, the initial powdery mildew development was completely inhibited after 27 days, in contrast to the continuous disease progression for the untreated control plants (Vechet et al. 2009). The long-lasting increased resistance to the disease suggests that treatments induced systemic acquired resistance in plants. Physcion, that is an active ingredient of the giant knotweed extract and of its commercial preparation Milsana^{TM}, has been reported to elicit plant defence-related mechanisms and especially to enhance expression of leaf-specific thionins in barley leaves (Ma et al. 2010).

A strong inhibitory effect of TTO, the EO extracted from *Melaleuca alternifolia*, on the growth of *B. graminis* f. sp. h*ordei* has been demonstrated in barley (Morcia et al. 2011a). The results obtained showed that even a single treatment with a spray solution containing TTO as low as 0.5 % is effective in the powdery mildew control and completely prevented the colonization of barley leaves (Fig. 14.2). Moreover, 24 h after the single treatment, the mycelium is necrotic,

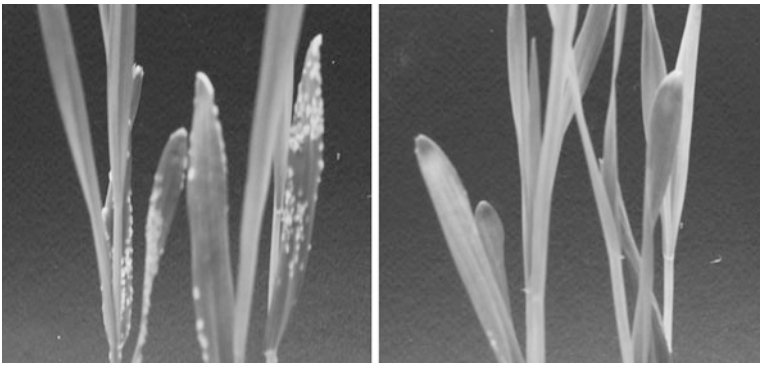

Fig. 14.2 Effect of 0.5 % TTO solution on powdery mildew colonies grown on barley leaves (barley cultivar Golden Promise, susceptible to the disease). *Left* barley control (i.e., not treated) plantlets 3 days after the inoculum with powdery mildew. *Right* barley treated (i.e., sprayed once with 0.5 % TTO solution) plantlets 3 days after the inoculum with powdery mildew

with drastic reduction of sporulating colonies and conidia and with the appearance of about 60 % of irregular conidia with fissured cell wall. No phytotoxic effects were observed on barley leaves treated with TTO solution and the growth of the sprayed leaves, monitored until the fourth leaf stage, did not differ from that of the control leaves.

14.3 EOs and Natural Compounds Against Fungi that Attack Cereals Mainly During Storage

The contamination of cereal products with harmful molds occurs even during storage, where specific conditions of temperature and humidity may contribute to implement their development. Undesirable microorganisms change the nutritive value of cereal-derived food and feed, are often responsible for off-flavor formation and produce toxins that can propagate along whole production chains. A growing body of control measures are continuously implementing in developed countries, but it is estimated that approximately 4/5 people living in developing countries are exposed to uncontrolled quantity of toxins (Williams et al. 2004).

Currently, synthetic preservatives, that prevent or retard pathogens growth, are used to control the dangerous microorganisms and, consequently, diseases caused by them. However, the massive use of synthetic preservatives can lead to the development of resistance in the pathogen populations (Dwivedi and Dubey 1993; Ahmed et al. 2011).

Alternative methods have therefore been studied in order to reduce the use of chemical treatments: natural extracts from plants appear to be a good alternative because these substances have the advantages of low toxicity and rapid degradation. EOs and their components can be postharvest bio-treatments due to their antibacterial, antiviral, antiparasitic, insecticidal, and fungicidal properties. In addition, most of EOs have been recognized as safe by the "United States Food and Drug Administration" (Burt 2004).

At present, many in vitro tests have shown that specific AMP extracts can limit spore germination and the development of mycotoxigenic spoilage fungi. EOs isolated from 18 plant species showed great variability in inhibition of in vitro growth of *A. flavus*, the main postharvest pathogen in storage cereals. Among the tested oils, those extracted from *Mentha arvensis* (0.5 mg ml^{-1}) is the most effective, causing complete inhibition of fungus growth and of aflatoxin B_1 production (Kumar et al. 2007).

Cardenas-Ortega et al. (2005) proposed the use of EO of *Chrysactinia mexicana*, a plant used in folk medicine, and piperitone, its major pure component, to protect cereal seeds and to inhibit *A. flavus* growth. *C. mexicana* oil has been demonstrated to be fungistatic and fungicidal at 1 and 1.25 mg/ml concentration, respectively, and piperitone caused a total fungal inhibition at 0.6 mg/ml in liquid medium. In vitro studies demonstrated even the potential food preservative capacity of *Ocimum*

gratissimum, *T. vulgaris,* and *Cymbopogon citratus* EOs against *A. flavus*, *Aspergillus fumigatus,* and *Fusarium moniliforme*, the most frequent mycotoxigenic fungi causing spoilage in African foodstuffs (Nguefack et al. 2004).

Cinnamomum camphora and *Alpina galanga* EOs are other two promising natural substances to control *A. flavus* proliferation: both the oils completely inhibited the fungal growth (at concentration of 1,000 ppm) and the aflatoxin B_1 production (at concentration of 500 ppm for *A. galanga* and 750 ppm for *C. camphora*). Moreover, the combination of the two oils was found superior in term of efficacy than the single ones. The mix showed in fact complete inhibition of the fungal development at 750 ppm and inhibition of aflatoxin production starting from 250 ppm. Hence, the combination of these two extracts resulted in a significant synergistic antifungal effect (Srivastava et al. 2008). These findings are in agreement with the assertion that most EOs are complex mixtures of many constituents and the interaction between volatile components may lead to synergistic phenomena that improve the potency of these natural extracts (Isman et al. 2011) . In Table 14.2 several examples of *in vivo* EOs antifungal activity on stored cereal seeds are reported.

14.4 Natural Substances Used in Maize Preservation and Storage

Maize is the second source of food and feed in the world (Fig. 14.1), representing in 2010 the 35 % of world cereal production quantity (Fig. 14.3) and the 24 % of the cereal world harvested area (Fig. 14.4). The protection against fungal contamination and their mycotoxins accumulation during storage is a very critical point for the safety of several agro-food chains because of the outstanding role of this cereal in human and animal nutrition. There is strong interest for viable methods alternative to the use of synthetic fungicides, so a large number of natural products have been tested for inhibitory effects against mycotoxigenic molds (Chulze 2010). In several works different types of plant extracts were tested against *A. flavus* and *F. verticillioides*, two pathogenic fungi associated with corn diseases as corn ear and kernel rot and with the production of aflatoxin and fumonisin mycotoxins during storage. These groups of mycotoxins are associated with devastating neurologic diseases as equine leukoencephalomalacia (Marasas et al. 1988; Giannitti et al. 2011) and porcine pulmonary edema (Harrison et al. 1990) and are implicated in the pathogenesis of esophageal liver cancer in humans (Ross et al. 1992).

Mentha viridis EO can reduce *A. flavus* aflatoxin production in stored corn: the use of 300 μL of mint in 100 g of corn is enough to prevent the growth of fungi and the mycotoxin synthesis (Gibriel et al. 2011). Bankole (1997) showed that this pathogenic fungus is inhibited by EOs obtained from *A. indica* seeds and leaves (50–100 ppm) and from *Morinda lucida* leaves (100 ppm). Moreover, the presence of extracts of these two Nigerian plants at concentration ranging from 500 to 1,000 ppm completely inhibited mycotoxin production in inoculated maize grain.

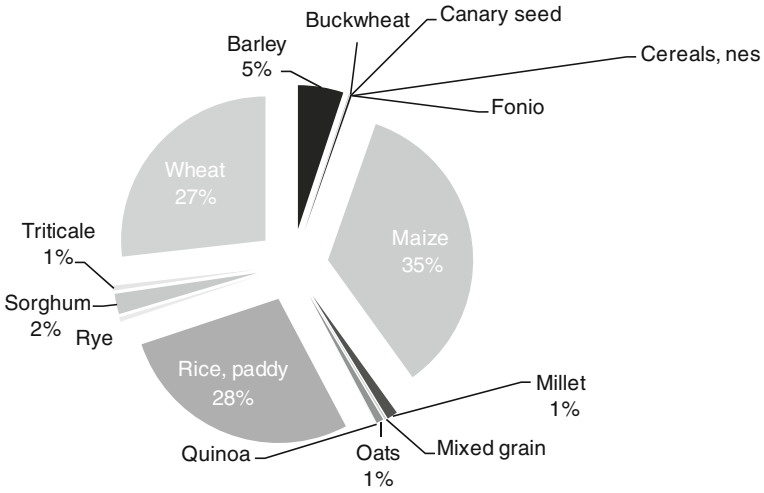

Fig. 14.3 Percentage of each cereal calculated on the world total cereal production quantity in 2010, according to FAOSTAT data publicly available at http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor

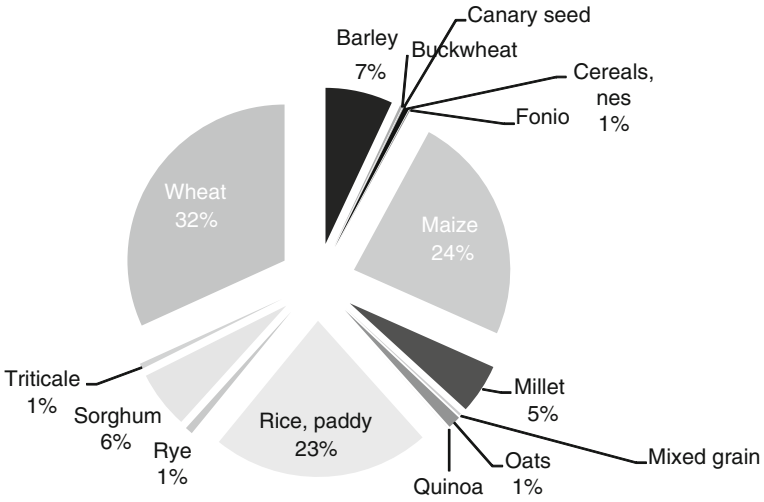

Fig. 14.4 Percentage of each cereal calculated on the world total cereal area in 2010, according to FAOSTAT data publicly available at http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor

Extracts of Cinnamomum zeylanicum (cinnamon), *Mentha piperita* (peppermint), *Ocimum basilicum* (basil), *Origanum vulgare* (origanum), *Teloxys ambrosioides* (the flavoring herb epazote), *Syzygium aromaticum* (clove), and *T. vulgaris* (thyme) were tested for maize kernel protection against *A. flavus*. All essential oils

caused a total inhibition of the fungus at concentration of 3–8 % and had not phytotoxic effects on corn germination and growth. A residual effect of cinnamon was detected after 4 weeks of kernel treatment (Montes-Belmont and Carvajal 1998).

500–1,000 mg of cinnamon, clove, lemon grass, oregano, and palmarosa EOs can be directly added to 1 kg of maize grains in order to prevent growth of *F. verticillioides* and *F. proliferatum* and their fumonisin B_1 production. Starting from these data, Velluti et al. (2003, 2004) proposed the use of these EOs and the control of the water activity to prevent the development of spoilage fungi in stored corn . These natural extracts were also tested, in controlled conditions, on maize seeds infected with *F. graminearum*: all oils (at 500 mg/kg^{-1} concentration) exerted a total prevention of deoxynivalenol at 0.995 a_w and 30°C and lemongrass, clove, and palmarosa of zearalenone at 0.995 a_w and 30°C (Marìn et al. 2004).

Fandohan et al. (2004) tested nine different extracts (*C. citratus*, *O. basilicum*, *O. gratissimum*, *Lantana camara*, *Eucalyptus citriodora*, *Clausena anisata*, *Melaleuca quinquenervia*, *Xylopia aethiopica*, *A. indica*), and selected *C. citratus*, *O. basilicum,* and *O. gratissimum* EOs as the most appropriate for *F. verticillioides* inhibition. The authors recommended to use 4.8 μL/g of these three extracts on stored corn stored in close conditions to prevent fumonisin production. However, they discouraged the use of EOs in open storage conditions, because oils are volatile compounds that are more likely to diffuse quickly in air, becoming ineffective in these specific storage situations.

14.5 Natural Substances Used in Wheat Preservation and Storage

Wheat is the most important source of food for human consumption (Fig. 14.1), representing in 2010 the 27 % of world cereal production quantity (Fig. 14.3) and the 32 % of the cereal world harvested area (Fig. 14.4). The control of mycotoxigenic fungi in both bread and durum wheat is essential because bread and pasta are consumed daily worldwide.

Paster et al. (1995) proposed the use of oregano and thyme EOs as an alternative to chemicals against fungi attacking stored wheat; these natural substances are efficient in controlling mycelia growth of *A. flavus*, *Aspergillus niger* and *Aspergillus ochraceus*.

EOs of 12 plants (thyme, cinnamon, marigold, spearmint, basil, quyssum, caraway, anise, ghafath, cinamone, chamomile, hazanbul) were tested at different concentration for the fungistatic/fungicidal activity against *A. flavus*, *Aspergillus parasiticus*, *A. ochraceus* and *F. moniliforme* on wheat grains. The extracts showed different capacity to inhibit fungi growth, and the effect was dose-dependent with the concentration of EOs. 2 % of thyme and anise oils was able to protect wheat by the fungi; moreover the use of thyme, cinnamon, anise, and spearmint at different concentration inhibited ochratoxin A (OTA) and fumonisin toxin production in inoculated grain (Soliman and Badeaa 2002).

Studies were carried out to evaluate the efficacy of thyme, clove, and cinnamon oil on the control of *Penicillium verrucosum* and *A. ochraceus* growth and OTA production in wheat grain under different water activity and temperature conditions of storage. 500 μg g^{-1} of all treatments were indispensable for 90 % reduction of fungal growth (in inoculated wheat grains over 28 day storage period) and were able to completely stop mycotoxin production when the conditions were 25°C and 0.90 a_w (Aldred et al. 2008).

14.6 Natural Substances Used in Rice Preservation and Storage

Rice is cultivated in more than a hundred countries and is the third source of food in the world (Fig. 14.1), representing in 2010 the 28 % of world cereal production quantity (Fig. 14.3) and the 23 % of the cereal world harvested area (Fig. 14.4).

To control *A. flavus* growth and aflatoxin B_1 production on rice, plant extracts of *Allium cepa*, *Allium sativum*, *Annona squamosa*, *A. indica*, *C. longa*, *Eucalyptus tereticornis*, *Ocimum sanctum*, *Pongamia glaberrima,* and *Syzigium aromaticum* have been tested. Among these nine plant extracts, *S.aromaticum* (5 g/kg) showed complete inhibition of fungus growth and aflatoxin B_1 production, while *C. longa*, *A. sativum,* and *O.sanctum* effectively inhibited *A.flavus* development (65–78 %) and aflatoxin B_1 production (72.2–85.7 %) at 5 g/kg concentration (Reddy et al. 2009).

Paranagama et al. (2003) suggested the use of lemongrass (*C. citratus*) EO to control fungal pest and mycotoxin production on stored rice. Lemongrass oil in the liquid culture was fungistatic and fungicidal against *A. flavus*, isolated from stored rice, at 0.6 and 1 mg ml^{-1} , respectively. Furthermore, aflatoxins were not detected in the liquid culture at concentration higher than 0.2 mg ml^{-1}. The fumigant toxicity assay of lemongrass oil demonstrated that the sporulation of *A. flavus* was completely inhibited at 2.8 mg ml^{-1} oil concentration. The clove and laurel EOs showed antifungal capacity on *Aspergillus candidus*, *A. niger*, *Fusarium culmorum,* and *Penicillium islandicum* (storage fungi or field fungi remaining alive during storage) isolated from rice (Magro et al. 2010).

14.7 Natural Substances Used in Sorghum Preservation and Storage

Today sorghum is one of the most important cereal crops in Africa and parts of Asia. Sorghum is the seventh most important source of food for human consumption (Fig. 14.1), representing in 2010 the 2 % of world cereal production quantity (Fig. 14.3) and the 6 % of the cereal world harvested area (Fig. 14.4).

Sorghum seeds in storage conditions are exposed to a wide typology of fungi and the use of natural extracts is of interest even in consideration of the traditional use of EOs in the areas of sorghum cultivation.

EOs of *O. basilicum*, *Cinnamomum cassia*, *Coriandrum sativum,* and *Laurus nobilis* at 1–5 % (v/v) concentration were tested for their activities in the control of *A. parasiticus* growth and its aflatoxins production. In vitro and in vivo experiment showed that 5 % of sweet basil is fungistatic and prevent the aflatoxins production (B_1 + G1) with a residual effect on stored sorghum grains for 32 days. In contrast, oils of *C. cassia* and *L. nobilis* stimulated the mycelia growth of the fungus in vitro but reduced the aflatoxin concentration of the fungus by 97.92 and 55.21 %, respectively. *C. sativum* oil did not have any effect on the mycelia growth and aflatoxin content. The combination of *C. cassia* and *O. basilicum* oils (2.5 % v/v) completely inhibited the fungal growth. Moreover, it was found that the addition of whole (5 % w/w) or ground (10 % w/w) dry *O. basilicum* leaves to 10 g sorghum seed are capable to control aflatoxins synthesis after 35 days storage period (Atanda et al. 2007).

14.8 Plant Bioactive Metabolites Involved in Cereal Protection: Their Mechanisms of Action and Impact on Fungal Cells

Up to now the identification of antifungal properties of EOs and plant metabolites has been mainly based on a simple screening among plant extracts and observations of their bioactivity (Lang and Buchbauer 2011). Despite the fact that many studies have been carried out on this topic, the EOs mechanisms of action are not yet deeply understood. A plethora of data indicates that the antimicrobial activity of EOs cannot be simply ascribed to one specific component neither can be considered as the sum of the actions of all the components. EOs act in a complex manner, due to the complexity of synergistic and antagonistic interactions among their major and minor components. The action of an EO is therefore different from the action of a mixture that contains only its major components. Because of the complexity of the problem, more information is available about the mechanisms of action of single EO components in comparison with whole oil.

One key factor related to EOs antifungal activity is surely their hydrophobicity. The terpenes that are present in several EOs can in fact attack the lipid structure of cell membranes, with the consequence that the permeability of cytoplasmic membranes increase, ions leave the cytoplasm, the cell wall is deteriorated and cell lysis can occur (Sanchez-Gonzalez et al. 2011). Not only cytoplasmic membrane can be affected by EOs treatment, but even mitochondrial ones, in which ionic channels and proton pumps are disrupted, with the consequent depolarization and permeabilization.

Table 14.2 Essential oils "in vivo" tested for their fungistatic/fungicidal effects and for their inhibitory actions against mycotoxins production in stored seeds inoculated with the indicated molds

Essential oils	Common name	Effective against	Host cereal	Storage periods	Inhibitory concentrations	References
Mentha viridis	Mint	*Aspergillus flavus*	Maize	7, 14 and 21 days	Fungistatic: ≥50 μL/100 g corn Fungicidal and antiflatoxigenic: ≥300 μL/100 g corn	Gibriel et al. (2011)
Azadirachta indica	Neem	*Aspergillus flavus*	Maize	10 days	Fungistatic: ≥50 ppm (seed oil) and ≥100 ppm (leaves oil) AntiflatoxigenicB_1: ≥500–1,000 ppm	Bankole (1997)
Morinda lucida	Brimstone tree	*Aspergillus flavus*	Maize	10 days	Fungistatic: ≥100 ppm AntiflatoxigenicB_1: ≥1,000 ppm	Bankole (1997)
Pimpinella anisum	Aniseed	*Aspergillus* section *Flavi*	Maize	11, 21 and 35 days	Fungistatic: 3,000 μg g^{-1}(35 days incubation period)	Bluma and Etcheverry (2008)
Peumus boldus	Boldo	*Aspergillus* section *Flavi*	Maize	11, 21 and 35 days	Fungistatic: 2,000 μg g^{-1} (35 days incubation period) AntiflatoxigenicB_1: 2,000–3,000 μg g^{-1}	Bluma and Etcheverry (2008)
Hedeoma multiflora	Mountain thyme	*Aspergillus* section *Flavi*	Maize	11, 21 and 35 days	Fungistatic: 2,000 μg g^{-1}(35 days incubation period) AntiflatoxigenicB_1: 2,000–3,000 μg g^{-1}	Bluma and Etcheverry (2008)
Syzygium aromaticum	Clove	*Aspergillus* section *Flavi*	Maize	11, 21 and 35 days	Fungistatic: 3,000 μg g^{-1}(35 days incubation period)	Bluma and Etcheverry (2008)
Lippia turbinate var. integrifolia	Poleo	*Aspergillus* section *Flavi*	Maize	11, 21 and 35 days	Fungistatic: 2,000 μg g^{-1}(35 days incubation period) AntiflatoxigenicB_1: 2,000–3,000 μg g^{-1}	Bluma and Etcheverry (2008)
Origanum vulgare	Oregano	*Fusarium verticillioides*	Maize	28 days	Fungistatic: 500–1,000 mg Kg^{-1}	Velluti et al. (2004)
Syzygium aromaticum	Clove	*Fusarium verticillioides*	Maize	28 days	Fungistatic: 500–1,000 mg Kg^{-1}	Velluti et al. (2004)

(continued)

Table 14.2 (continued)

Essential oils	Common name	Effective against	Host cereal	Storage periods	Inhibitory concentrations	References
Cinnamomum zeylanicum	Cinnamon	*Fusarium verticillioides*	Maize	28 days	Fungistatic: 500–1,000 mg Kg^{-1}	Velluti et al. (2004)
Cymbopogon citrates	Lemongrass	*Fusarium verticillioides*	Maize	28 days	Fungistatic: 500–1,000 mg Kg^{-1}	Velluti et al. (2004)
Cymbopogon martini	Palmarosa	*Fusarium verticillioides*	Maize	28 days	Fungistatic: 500–1,000 mg Kg^{-1}	Velluti et al. (2004)
Origanum vulgare	Oregano	*Fusarium proliferatum*	Maize	28 days	Fungistatic: 500–1,000 mg Kg^{-1} (20 °C and 0.995 or 0.950 a_w; 30 °C and 0.995 a_w)	Velluti et al. (2003)
Syzygium aromaticum	Clove	*Fusarium proliferatum*	Maize	28 days	Fungistatic: 500–1,000 mg Kg^{-1} (20 °C, 0.995 or 0.950 a_w)	Velluti et al. (2003)
Cinnamomum zeylanicum	Cinnamon	*Fusarium proliferatum*	Maize	28 days	Fungistatic: 1,000 mg Kg^{-1} (20 °C and 0.995 or 0.950 a_w; 30 °C and 0.995 a_w)	Velluti et al. (2003)
Cymbopogon citrates	Lemongrass	*Fusarium proliferatum*	Maize	28 days	Fungistatic: 500 (20 °C and 0.995 or 0.950 a_w; 30 °C and 0.995 a_w)	Velluti et al. (2003)
Cymbopogon martini	Palmarosa	*Fusarium proliferatum*	Maize	28 days	Fungistatic: 500 (20 °C, 0.995 or 0.950 a_w)	Velluti et al. (2003)
Cymbopogon citrates	Lemongrass	*Fusarium verticillioides*	Maize	21 days and 6 weeks	Fungicidal: 8 µl/g Antifumonisin: 4.8 µl/g	Fandohan et al. (2004)
Ocimum basilicum	Basil	*Fusarium verticillioides*	Maize	21 days and 6 weeks	Fungicidal: 6.4 µl/g Antifumonisin: 4.8 µl/g	Fandohan et al. (2004)
Ocimum gratissimum	Basil	*Fusarium verticillioides*	Maize	21 days and 6 weeks	Fungicidal: 4.8 µl/g Antifumonisin: 4.8 µl/g	Fandohan et al. (2004)
Cinnamomum zeylanicum	Cinnamon	*Fusarium graminearum*	Maize	28 days	AntiDON: 500 mg/Kg^{-1} (0.995 a_w and 30 °C)	Marìn et al. (2004)

(continued)

Table 14.2 (continued)

Essential oils	Common name	Effective against	Host cereal	Storage periods	Inhibitory concentrations	References
Syzygium aromaticum	Clove	*Fusarium graminearum*	Maize	28 days	AntiZEA: 500 mg/Kg^{-1} (0.950 a_w and 30 °C) AntiDON: 500 mg/Kg^{-1} (0.995 a_w and 30 °C)	Marìn et al. (2004)
Origanum vulgare	Oregano	*Fusarium graminearum*	Maize	28 days	AntiDON: 500 mg/Kg^{-1} (0.995 a_w and 30 °C)	Marìn et al. (2004)
Cymbopogon martini	Palmarosa	*Fusarium graminearum*	Maize	28 days	AntiZEA: 500 mg/Kg^{-1} (0.950 a_w and 30 °C) AntiDON: 500 mg/Kg^{-1} (0.995 a_w and 30 °C)	Marìn et al. (2004)
Cymbopogon citratus	Lemongrass	*Fusarium graminearum*	Maize	28 days	AntiZEA: 500 mg/Kg^{-1} (0.950 a_w and 30 °C) AntiDON: 500 mg/Kg^{-1} (0.995 a_w and 30 °C)	Marìn et al. (2004)
Pimpinella anisum	Anise	*A. flavus*, *A. parasiticus*, *A. ochraceus* and *Fusarium moniliforme*	Wheat	2, 4, 8 weeks	Fungicidal: 500 ppm	Soliman and Badeaa (2002)
Carum carvi	Caraway	*A. flavus*, *A. parasiticus*, *A. ochraceus* and *Fusarium moniliforme*	Wheat	2, 4, 8 weeks	Fungicidal: 2,000–3,000 ppm	Soliman and Badeaa (2002)
Foeniculum vulgare	Fennel	*A. flavus*, *A. parasiticus*, *A. ochraceus* and *Fusarium moniliforme*	Wheat	2, 4, 8 weeks	Fungicidal: 2,000–3,000 ppm	Soliman and Badeaa (2002)
Thymus vulgaris	Thyme	*A. flavus*, *A. parasiticus*, *A. ochraceus* and *Fusarium moniliforme*	Wheat	2, 4, 8 weeks	Fungicidal: 500 ppm	Soliman and Badeaa (2002)

(continued)

Table 14.2 (continued)

Essential oils	Common name	Effective against	Host cereal	Storage periods	Inhibitory concentrations	References
Mentha viridis	Spearmint	*A. flavus*, *A. parasiticus*, *A. ochraceus* and *Fusarium moniliforme*	Wheat	2, 4, 8 weeks	Fungicidal: 3,000 ppm	Soliman and Badeaa (2002)
Ocimum basilicum	Basil	*A. flavus*, *A. parasiticus*, *A. ochraceus and Fusarium moniliforme*	Wheat	2, 4, 8 weeks	Fungicidal: 3,000 ppm	Soliman and Badeaa (2002)
Matricaria chamomilla	Chamomile	*A. flavus*, *A. parasiticus*, *A. ochraceus* and *Fusarium moniliforme*	Wheat	2, 4, 8 weeks	Fungistatic: 3,000 ppm (90–95 % reduction of fungi growth)	Soliman and Badeaa (2002)
Calendula officinalis	Marigold	*A. flavus*, *A. parasiticus*, *A. ochraceus* and *Fusarium moniliforme*	Wheat	2, 4, 8 weeks	Fungicidal: 2,000 ppm	Soliman and Badeaa (2002)
Achillea millefolium	Hazambul	*A. flavus*, *A. parasiticus*, *A. ochraceus* and *Fusarium moniliforme*	Wheat	2, 4, 8 weeks	Fungistatic: 500 ppm	Soliman and Badeaa (2002)
Achillea		*fragrantissima*	Qyssum	*A. flavus*,	*A. parasiticus*, *A. ochraceus* and *Fusarium moniliforme*	Wheat
2, 4, 8 weeks	Fungicidal:	3,000 ppm	Soliman and	Badeaa (2002)		
Agrimonia eupatoria	Ghafath	*A. flavus*, *A. parasiticus*, *A. ochraceus* and *Fusarium moniliforme*	Wheat	2, 4, 8 weeks	Fungistatic: 500 ppm	Soliman and Badeaa (2002)
Cinnamomum zeylanicum	Cinnamon	*A. flavus*, *A. parasiticus*, *A. ochraceus* and *Fusarium moniliforme*	Wheat	2, 4, 8 weeks	Fungicidal: 500 ppm	Soliman and Badeaa (2002)

(continued)

Table 14.2 (continued)

Essential oils	Common name	Effective against	Host cereal	Storage periods	Inhibitory concentrations	References
Syzygium aromaticum	Clove	*P. verrucosum* and *A. ochraceus*	Wheat	28 days	Fungistatic: 200–300 μg g^{-1} (50 % inhibition) Antiochratoxin: 500 μg g^{-1}(at 0.9 a_w and 25 °C)	Aldred et al. (2008)
Cinnamomum zeylanicum	Cinnamon	*P. verrucosum* and *A. ochraceus*	Wheat	28 days	Fungistatic: 200–300 μg g^{-1} (50 % inhibition) Antiochratoxin: 500 μg g^{-1}(at 0.9 a_w and 25 °C)	Aldred et al. (2008)
Thymus vulgaris	Thyme	*P. verrucosum* and *A. ochraceus*	Wheat	28 days	Fungistatic: 200–300 μg g^{-1} (50 % inhibition) Antiochratoxin: 500 μg g^{-1}(at 0.9 a_w and 25 °C)	Aldred et al. 2008)
Syzygium aromaticum,	Clove	*Aspergillus flavus*	Rice	5 days	Fungicidal 5 g/kg Antiaflatoxin: 5 g/kg	Reddy et al. (2009)
Curcuma longa	Curcumin	*Aspergillus flavus*	Rice	5 days	Fungistatic: 5 g/kg (68 % inhibition) Antiaflatoxin: 5 g/kg (72.2 % reduction)	Reddy et al. (2009)
Allium sativum	Garlic	*Aspergillus flavus*	Rice	5 days	Fungistatic: 5 g/kg (65 % inhibition) Antiaflatoxin: 5 g/kg (75 % reduction)	Reddy et al. (2009)
Ocimum sanctum	Holy basil	*Aspergillus flavus*	Rice	5 days	Fungistatic: 5 g/kg (78 % inhibition) Antiaflatoxin: 5 g/kg (85.7 % reduction)	Reddy et al. (2009)
Ocimum basilicum	Basil	*Aspergillus parasiticus*	Sorghum	8, 16, 24, 32, 40 days	Fungistatic: 5 % oil treatment on 120 grains (100 % healthy kernels for 32 days) Antiaflatoxin: 50 mg/g of leaves reduced aflatoxin (B_1 + G1) by 74.47 % (whole leaves) and 72.35 % (ground leaves); 100 mg/g of leaves reduced aflatoxin (B_1 + G1) by 90.60 % (whole leaves) and 89.05 % (ground leaves)	Atanda et al. (2007)

(continued)

Table 14.2 (continued)

Essential oils	Common name	Effective against	Host cereal	Storage periods	Inhibitory concentrations	References
Cinnamomum cassia	Cinnamon, cassia	*Aspergillus parasiticus*	Sorghum	8, 16, 24, 32, 40 days	Fungistatic: 5 % oil treatment on 120 grains (100 % healthy Kernels for 16 days)	Atanda et al. (2007)
Coriandrum sativum	Coriander	*Aspergillus parasiticus*	Sorghum	8, 16, 24, 32, 40 days	Fungistatic: 5 % oil treatment on 120 grains (100 % healthy Kernels for 16 days)	Atanda et al. (2007)
Laurus nobilis	Laurel	*Aspergillus parasiticus*	Sorghum	8, 16, 24, 32, 40 days	Fungistatic: 5 % oil treatment on 120 grains (100 % healthy Kernels for 16 days)	Atanda et al. (2007)

At cellular level, this attack to the lipophilic structure results in several morpho-physiological alterations. In *B. cinerea* EOs can inhibit spore germination and germ tube elongation. SEM characterization revealed morphological degeneration of hyphae, cytoplasmic coagulation, vacuolization, hyphal shriveling, and protoplasts leakage (Soylu et al. 2010). *A. heterotropoides* EO causes in several plant fungal pathogens loss of micro-organellar membrane integrity (Dan et al. 2010) explained by the interactions between cyclic hydrocarbons of the oil and hydrophobic part of the fungal cell. In this oil, methyleugenol is the main component and is responsible for damages to membrane, mitochondria and lomasome integrity, and changes in electron density.

The mechanism of action of *M. alternifolia* EO (TTO) at cellular level, involves both the loss of membrane integrity accompanied by the release of intracellular material and the inhibition of cellular respiration, with the consequent inability to maintain homeostasis associated with changes in cell morphology (Carson et al. 2006). Its biological effects have been deeply evaluated in model fungi, such as *Saccharomyces cerevisiae*, that offers advanced toolkits of experimental approaches (Straede and Heinisch 2007). *S. cerevisiae* has a rigid cell wall whose synthesis is controlled by a highly conserved MAP kinase signal transduction cascade. In stress conditions, a set of sensors activate, through this cascade, the transcription factor *Rlm1*, which governs expression of many genes encoding enzymes of cell wall biosynthesis. A set of yeast strains transformed with reporter constructs which link activation of a hybrid, *Rlm1–lexA*, by the MAP kinase *Mpk1/Slt2* to the expression of the bacterial *lacZ* gene was used to have better insight into molecular response to TTO. The authors found a clear dose-dependent increase in β-galactosidase activities, demonstrating an activation of cell integrity signaling by TTO.

Rao et al. (2010) studied the inhibitory effect of oregano oil on *S. cerevisiae* cells and found that single terpenoid phenol contributes at cellular and molecular level. The data showed that the monoterpenoid phenol carvacrol is the most potent compound in oregano oil and disrupts ion homeostasis in yeast. After the addition of carvacrol, in a dose-dependent way, Ca^{2+} elevation is observed, consistent with an influx of Ca^{2+} from extracellular medium, followed by sequestration into vacuoles or efflux from cell. The structural isomer thymol causes a similar Ca^{2+} burst, whereas eugenol is less effective. The toxicity of carvacrol or thymol is more evident in yeast *vma2Δ* mutant, in which is lacking a functional vacuolar H+ pump and therefore the clearance of cytosolic Ca^{2+} is severely impaired. Accordingly to these results, the transcriptional response to carvacrol in yeast resembles the Ca^{2+} stress, with up-regulation of genes involved in energy-handling pathways, stress response/signaling, and drug efflux mechanisms. On the contrary, the genes involved in nucleic acid metabolism and RNA synthesis are severely down-regulated, consistently with cessation of growth. Changes in pH followed the Ca^{2+} burst, arguing against a simple mechanism of membrane lesion.

The down-regulation of genes involved in several aspects of RNA metabolism and ribosome biogenesis has been found even in thymol-treated *S. cerevisiae* cells, confirming that this energy-demanding process is tightly coordinated with environmental growth conditions (Bi et al. 2009). These authors determined the

transcriptional response of *S. cerevisiae* upon exposure to thymol, taking a whole-genome view to elucidate its mechanism of action. Several important genes involved in different pathways were affected by thymol treatment. In particular, is strongly inhibited the metabolism of thiamin, that works as cofactor for enzymes of carbohydrate and amino acids pathways. On the contrary, genes involved in phospholipid synthesis are strongly induced, together with myo-inositol transporter genes involved in maintaining the cell membrane and cell wall integrity. These results confirmed that this monoterpene acts by primary lesion of the membrane. Even genes related to sulfur assimilation category are up-regulated and their elevated transcripts levels could have the effect of increasing phospholipid biosynthesis. Specific transcription factors are modulated by thymol, like *Msn2p* and *Msn4p* factors that control stress genes, *Pdr1p,* and *Pdr3p* factor that regulate the multidrug resistance response, *TBP* factor involved in induction of TATA-binding proteins and *AFT1* factor that regulates the iron uptake.

Of particular interest for the safety of several food and feed production chains, is the knowledge of the molecular mechanisms by which some EOs act as inhibitors of mycotoxins production by Fusaria. Yaguchi et al. (2009) isolated precocenes and piperitone from the EOs of *Matricaria recutita* and *Eucalyptus dives*, respectively, as specific inhibitors of the production of 3-acetyldeoxynivalenol, a biosynthetic precursor of deoxynivalenol. These authors demonstrated that the inhibitory activities against 3-acetyldeoxynivalenol and deoxynivalenol production happen together with the inhibition of the transcription of genes encoding proteins required for deoxynivalenol biosynthesis. When precocene II and piperitone were added to *Fusarium* strains cultured in liquid medium, a dose-dependent decrease or suppression of enzyme-encoding genes *Tri4* and *Tri 5* and of their regulators *Tri6* and *Tri10* has been observed. TRI10 is the first key regulator of the trichothecene biosynthetic pathway and the upstream events have not yet been clarified. The molecular targets of piperitone and precoceneII are therefore present in an unknown early step of DON biosynthetic route. To better elucidate this key point, Tsuyuki et al. (2011) started from the knowledge that in some fungal species, CoCl2 affects the regulatory system of ergosterol biosynthesis, inducing the expression of fungal genes homologous to sterol regulatory element-binding protein (SREBP) and SCAP (SREBP cleavage-activating protein), which leads to further up-regulation of the expression of many enzyme genes involved in ergosterol biosynthesis. Because in *F. graminearum*, the mevalonate pathway is used both for trichothecene and for ergosterol biosynthesis, the expectation was that $CoCl_2$ affects the regulatory system of not only ergosterol production but also that of trichothecene production. The $CoCl_2$ presence in liquid culture strongly enhanced the trichothecene production, but not the ergosterol production. At transcriptional level, the $CoCl_2$ presence strongly up-regulates genes encoding trichothecene biosynthetic proteins, ergosterol biosynthetic enzymes, and enzymes involved in the mevalonate pathway. However, if precocene II is added, the suppression of the effects of cobalt chloride on *Tri4*, *Tri6*, *HMGS*, and *HMGR*, is observed, but no effect is observed on *erg3* and *erg25* levels. Precocenes I and II, major constituents of *A. conyzoides* EO, completely

inhibit even aflatoxin B_1 production by *A. flavus* and cause a retarded fungal growth (Nogueira et al. 2010). The cellular targets are the plasma and mitochondrial membranes: the destruction of mitochondria explains the loss of aflatoxins that are originated from beta-oxidation of short chain fatty acids in these organelles.

Spiro ethers from *M. recutita* have inhibitory effects on trichothecene biosynthesis, with a different molecular mechanism, because efficiently inhibit TRI4, the key P450 monooxygenase involved in the early step of the biosynthetic pathway (Yoshinari et al. 2008). Spiro ethers have strong inhibitory effect even on aflatoxin biosynthesis in a dose-dependent manner affecting the pathway from OMST to aflatoxin G_1 and targeting specifically CypA, a P450 monooxygenase enzyme (Yoshinari et al. 2008). Other phenylpropanoids, apiol, and dillapiole, may inhibit AFG1 biosynthesis via inhibition of the P450-dependent monooxygenase CypA of cytochromes (Razzaghi-Abyaneh et al. 2007).

Finally, thymol and carvacrol, targeting the mitochondrial oxidative stress defense system, inhibit in a dose-dependent manner the aflatoxins G_1 production in *A.parasiticus* (Kim et al. 2006).

14.9 Plant Bioactive Metabolites Involved in Cereal Protection: Their Mechanisms of Action and Impact on Plant Cells

Aromatic plants have long been known to emit volatile growth inhibitors or to contain EOs responsible for allelopathic interactions that can inhibit seed germination and plant growth, cause morphological alterations in plant seedlings, and inhibit the root elongation. Starting from the idea of using EOs as natural fungicides on crop the importance of the knowledge of their cellular and molecular mechanisms of action in plants has been realized.

Monoterpenes like camphor and menthol at high atmospheric concentrations enhance the transpiration in Arabidopsis leaves causing a dramatic increase of the stomata aperture, followed by extreme swelling and break down of the protoplasts (Schulz et al. 2007). The lipophilic layer of cuticular waxes in the leaf surface is the target of this allelopathic attack. The block of stomatal closure is accompanied by changes in cytoskeleton that are directly involved in stomatal movements. Transient changes in the expression of genes involved in wax production, stress response, and stomatal opening are observed (Krieg et al. 2010). The final hypothesis is that the exposure to high concentrations of monoterpenes led to irreversible damages in the plant, whereas low concentrations have the potential to strengthen the plant fitness. 1,8-cineole not only inhibits cell elongation in tobacco protoplasts, but affects a wide spectrum of cellular activities, including starch accumulation, in a non-specific manner. Several works have demonstrated that mitochondrial membranes are one of the primary target of monoterpenes: the

consequent inhibition of mitochondrial energy metabolism can result in disturbances in a wide range of physiological and biochemical processes (Yoshimura et al. 2011).

Kapros and McDaniel (2009) have demonstrated that tea tree oil has a major effect on membrane permeability not only in bacteria and fungi, but also in tobacco cells. These authors conclude that *Melaleuca* oils' mode of action at the cellular level can explain the inhibition of germination and seedling growth observed in nature due to the action of the oil.

A different mechanism of action is shown by citral, present in several EOs, constituted by a mixture of the two monoterpenes geranial and neral and of great interest as food additives (Ait-Ouazzou et al. 2011a). In *Arabidopsis* seedlings Chaimovitsh et al. (2010) demonstrated that citral disrupts microtubules but not actin fibers. At lower concentrations citral affects cell division by disrupting mitotic microtubules and cell plates, whereas at higher concentrations inhibits cell elongation by disrupting cortical microtubules (Chaimovitsh et al. 2011).

14.10 Conclusions and Future Perspectives

A great body of literature is available on EOs antifungal activities, however, no obvious linkage is apparent between their chemical composition and their effects. Some EOs are in fact rich in non-phenolic terpenes, whereas others are predominated by phenolic monoterpenes. Therefore, the finding of new strategies for crop protection and for post-harvest control based on natural products is now moving toward a more integrated approach involving disciplines such as molecular and cellular biology, proteomics, transcriptomics, and bioinformatics. The rationale behind this innovative approach is that a better knowledge of the molecular targets and mechanisms of actions of plant metabolites at cellular level can optimize their utilization, alone or in combination, and maximize the antifungal efficacy. As an example of this strategy, in the work of Kim et al. (2010) it has been demonstrated that thymol acts as potent chemosensitizing agent that, when co-applied with the antifungal azole drugs, gives the complete inhibition of fungal growth at dosages far lower than the drugs alone. The perspectives of EOs application in cereal protection require even that the screening of natural molecules will be based on high-throughput tools, already developed in the field of human pathogens (Kuete et al. 2011). Moreover, great interest is in the design of combined processes for cereal preservation, based on combination of EOs and natural metabolites with other traditional or emerging preservation methods in order to achieve synergistic antimicrobial effects (Ait-Ouazzou 2011a). Finally, a very interesting route of application has been developed, in the field of human pathogens, by Makarovsky et al. (2011) with the design and synthesis of novel hybrid-silica nanoparticles (NPs) containing antimicrobials covalently linked within the inorganic matrix for its controlled and slow release. The perspectives are the development of such

formulations for EOs components to be delivered to cereal fungi for much improved pathogen control/antimicrobial and antifungal effectiveness.

References

Ahmed A, Randhawa MA, Yusuf MJ, Khalid N (2011) Effect of processing on pesticide residues in food crops—a review. J Agric Res 49(3):379–390

Ait-Ouazzou A, Cherrat L, Somolinos M, Lorán S, Rota C, Pagán R (2011a) The antimicrobial activity of hydrophobic essential oil constituents acting alone or in combined processes. Inn Food Sci Emerging Technol 12:320–329

Ait-Ouazzou A, Lorán S, Rota C, Bakalli M, Laglaoui A, Herrera A, Pagán R, Conchello P (2011b) Antimicrobial activity of *Thymus algeriensis*, *Eucalyptus globulus* and *Rosmarinus officinalis* essential oils from Morocco. J Sci Food Agric 91:2643–2651

Aldred D, Cairns-Fuller V, Magan N (2008) Environmental factors affect efficacy of some essential oils and resveratrol to control growth and ochratoxin A production by *Penicillium verrucosum* and *Aspergillus westerdijkiae* on wheat grain. J Stored Prod Res 44:341–346

Al-Reza SM, Rahman A, Ahmed Y, Kang SC (2010) Inhibition of plant pathogens in vitro and in vivo with essential oil and organic extracts of *Cestrum nocturnum* L. Pesticide Biochem Physiol 96:86–92

Atanda OO, Akpan I, Oluwafemi F (2007) The potential of some spice essential oils in the control of *A. parasiticus* CFR 223 and aflatoxin production. Food Control 18:601–607

Bajpai VK, Kang SC (2010) Antifungal activity of leaf essential oil and extracts of *Metasequoia glyptostroboides* Miki ex Hu. J Am Oil Chem Soc 87:327–336

Bankole SA (1997) Effect of essential oil from two Nigerian medicinal plants (*Azadirachta indica* and *Morinda lucida*) on growth and aflatoxin B_1 production in maize grain by a toxigenic *Aspergillus flavus*. LAM 24:190–192

Bennett RN, Wallsgrove RM (1994) Secondary metabolites in plant defence mechanisms. New Phyt 127(4):617–633

Bi X, Guo N, Jin J, Liu, Feng H, Shi J, Xiang H, Wu X, Dong J, Hu H, Yan S, Yu C, Wang X, Deng X, Yu L (2009) The global gene expression profile of the model fungus *Saccharomyces cerevisiae* induced by thymol. J Appl Microbiol 108:712–722

Bluma R, Etcheverry M (2008) Application of essential oils in maize grain: impact on *Aspergillus* section *Flavi* growth parameters and aflatoxin accumulation. Food Microbiol 25:324–334

Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods—a review. Int J Food Microbiol 94:223–253

Cardenas-Ortega NC, Zavala-Sanchez MA, Aguirre-Rivera JR, Perez-Gonzalez C, Perez-Gutierrez S (2005) Chemical composition and antifungal activity of essential oil of *Chrysactinia mexicana* gray. J Agric Food Chem 53:4347–4349

Carson CF, Hammer KA, Riley TV (2006) *Melaleuca alternifolia* (tea tree) oil: a review of antimicrobial and other medicinal properties. Clin Microbiol Rev 19:50–62

Cawood ME, Pretorius JC, van der Westhuizen AJ, Pretorius ZA (2010) Disease development and PR-protein activity in wheat (*Triticum aestivum*) seedlings treated with plant extracts prior to leaf rust (*Puccinia triticina*) infection. Crop Prot 29:1311–1319

Chaimovitsh D, Abu-Abied M, Belausov E, Rubin B, Dudai N, Sadot E (2010) Microtubules are an intracellular target of the plant terpene citral. The Plant J 61:399–408

Chaimovitsh D, Rogovoy O, Altshuler O, Belausov E, Abu-Abied M, Rubin B, Sadot E, Dudai N (2011) The relative effect of citral on mitotic microtubules in wheat roots and BY2 cells. Plant Biol. doi:10.1111/j.1438-8677.2011.00511.x

Chulze SN (2010) Strategies to reduce mycotoxin levels in maize during storage: a review. Food Add Contam 27(5): 651-657

Dan Y, Liu H-Y, Gao W-W, Chen S-L (2010) Activities of essential oils from *Asarum hetrotropoides* var. *mandshuricum* against five phytopathogens. Crop Prot 29:295–299

Dwivedi SK, Dubey NK (1993) Potential use of the essential oil of *Trachyspermum ammi* against seed-borne fungi of Guar (*Cyamopsis tetragonoloba* L. (Taub.)). Mycopathologia 121:101–104

Fandohan P, Gbenou JD, Gnonlonfin B, Hell K, Marasas WFO, Wingfield MJ (2004) Effect of essential oils on the growth of *Fusarium verticillioides* and fumonisin contamination in corn. J Agri Food Chem 52:6824–6829

Giannitti F, Diab SS, Pacin AM, Barrandeguy M, Larrere C, Ortega J, Uzal FA (2011) Equine leukoencephalomalacia (ELEM) due to fumonisins B_1 and B_2 in Argentina. Pesquisa Veterinária Brasileira 31(5):407–412

Gibriel YAY, Hamza AS, Gibriel AY, Mohsen SM (2011) In vivo effect of mint (*Mentha viridis*) essential oil on growth and aflatoxin production by *Aspergillus flavus* isolated from stored corn. J Food Saf 3:445–451

Harrison LR, Colvin BM, Greene JT, Newman LE, Cole JR (1990) Pulmonary edema and hydrothorax in swine produced by fumonisin B_1 a toxic metabolite of *Fusarium moniliforme*. J Vet Diagn Inv 2:217–221

Isman MB, Miresmailli S, Machial C (2011) Commercial opportunities for pesticides based on plant essential oils in agriculture, industry and consumer products. Phytochem Rev 10(2):197–204

Kapros T, McDaniel SC (2009) Cytotoxicity of tea tree oil in tobacco cells. Allelopathy J 23 Print ISSN: 0971-4693. Online ISSN: 0973-5046

Kim JH, Campbell BC, Mahoney N, Chan KL, Molineaux RJ, Balajee A (2010) Augmenting the activity of antifungal agents against Aspergilli using structural analogues of benzoic acid as chemosensitizing agents. Fungal Biol 114:817–824

Kim JH, Mahoney N, Chan K, Molyneaux KL, Campbell BC (2006) Controlling food contaminating fungi by targeting antioxidant stress-response system with natural phenolic compounds. Appl Microbiol Biotechnol 70:735–739

Krieg B, Jansen M, Hahn K, Peisker H, Samajova O, Beck M, Braun S, Ulbrich A, Baluska F, Schulz M (2010) Cyclic monoterpenes mediated modulations of *Arabidopsis thaliana* phenotype. Plant Signal Behav 5(7):832–838

Kuete V, Alibert-Franco S, Eyong KO, Ngameni B, Folefoc GN, Nguemeving JR, Tangmouo JG, Fotso GW, Komguem J, Ouahouo BM, Bolla JM, Chevalier J, Ngadjui BT, Nkengfack AE, Pagès JM (2011) Antibacterial activity of some natural products against bacteria expressing a multidrug-resistant phenotype. Int J Antimicrob Ag 37(2):156–161

Kumar A, Shukla R, Singh P, Anuradha, Dubey NK (2010) Efficacy of extract and essential oil of *Lantana indica* Roxb. against food contaminating moulds and aflatoxin B_1 production. Int J Food Sci Technol 45:179–185

Kumar R, Dubey NK, Tiwari OP, Tripathi YB, Sinha KK (2007) Evaluation of some essential oils as botanical fungitoxicants for the protection of stored food commodities from fungal infestation. J Sci Food Agric 87:1737–1742

Lang G, Buchbauer G (2011) A review on recent research results (2008–2010) on essential oils as antimicrobials and antifungals. Flavour Frag J 27(1):13–39

Liu HY, Gao WW, Fan Y, Chen SL (2007) Inhibitory effect of essential oil from *Asarum heterotropoides* Fr. Schmidt var. *mandshuricum* (Maxim.) Kitag against plant pathogenic fungi. Acta Phytopathol Sin 37:95–98

Ma X, Yang X, Zeng F, Yang L, Yu D, Ni H (2010) Physcion, a natural anthraquinone derivative, enhances the gene expression of leaf-specific thionin of barley against *Blumeria graminis*. Pest Manag Sci 66:718–724

Makarovsky I, Boguslavsky Y, Alesker M, Lellouche J, Banin E, Lellouche JP (2011) Novel triclosan-bound hybrid-silica nanoparticles and their enhanced antimicrobial properties. Advanced Func Mat 21(22):4295–4304

Magro A, Matos O, Bastos M, Carolino M, Lima A, Mexia A (2010) The use of essential oils to protect rice from storage fungi. In: 10th international working conference on stored product protection 542 Julius-Kühn-Archiv, p 425

Marasas WFO, Kellerman TS, Gelderblom WCA, Coetzer JAW, Thiel PG, van der Lugt JJ (1988) Leukoencephalomalacia in a horse induced by fumonisin B_1 isolated from *Fusarium moniliforme*. Onderstepoort J Vet Res 55:197–203

Marìn S, Velluti A, Ramos AJ, Sanchis V (2004) Effect of essential oils on zearalenone and deoxynivalenol production by *Fusarium graminearum* in non-sterilized maize grain. Food Microbiol 21:313–318

Montes-Belmont R, Carvajal M (1998) Control of *Aspergillus flavus* in maize with plant essential oils and their components. J Food Prot 61:616–619

Morcia C, Spini M, Malnati M, Stanca Am, Terzi V (2011a) Essential oils and their components for the control of phytopathogenic fungi that affect plant health and agri-food quality and safety. In: Rai M, Chikindas M (eds) Natural antimicrobials for food safety and food quality. CABI Press, Oxford, pp 224–241, ISBN 978-1-84593-769-0

Morcia C, Malnati M, Terzi V (2012) *In vitro* antifungal activity of terpinen-4-ol, eugenol, carvone, 1,8-cineole (eucalyptol) and thymol against mycotoxigenic plant pathogens. Food Add Contam 29(3):415-422

Nguefack J, Leth V, Amvam Zollo PH, Mathur SB (2004) Evaluation of five essential oils from aromatic plants of Cameroon for controlling food spoilage and mycotoxin producing fungi. Int J Food Microbiol 94:329–334

Nogueira JHC, Goncalez E, Galletti SR, Facanali R, Marques MO, Felicio JD (2010) *Ageatum conyzoides* essential oil as aflatoxin suppressor of *Aspergillus flavus*. Int J Food Microbiol 137:55–60

Paranagama PA, Abeysekera KHT, Abeywickrama K, Nugaliyadde L (2003) Fungicidal and anti-aflatoxigenic effects of the essential oil of *Cymbopogon citratus* (DC.) Stapf. (lemongrass) against *Aspergillus flavus* Link. isolated from stored rice. LAM 37:86–90

Paster N, Menasherov M, Ravid U, Juven B (1995) Antifungal activity of oregano and thyme essential oils applied as fumigants against fungi attacking stored grain. J Food Sci 58:81–85

Rao A, Zhang Y, Muend S, Rao R (2010) Mechanism of antifungal activity of terpenoid phenols resembles calcium stress and inhibition of the TOR pathway. Antimicrob Agents Chemother 54(12):5062–5069

Razzaghi-Abyaneh M, Yoshinari T, Shams-Ghahfarokhi M, Rezaee MB, Nagasawa H, Sakuda S (2007) Dillapiol and apiol as specific inhibitors of the biosynthesis of aflatoxin G_1 in *Aspergillus parasiticus*. Biosci Biotechnol Biochem 71:2329–2332

Reddy KRN, Reddy CS, Muralidharan K (2009) Potential of botanicals and biocontrol agents on growth and aflatoxin production by *Aspergillus flavus* infecting rice grains. Food Control 20:173–178

Ross RK, Yu MC, Henderson BE, Yuan J-M, Qian G-S, Tu J-T, Gao Y-T, Wogan GN, Groopman JD (1992) Urinary aflatoxin biomarkers and risk of hepatocellular carcinoma. The Lancet 339(8799):943–946

Sanchez-Gonzalez L, Vargas M, Gonzalez-Martinez C, Chiralt A, Chafer M (2011) Use of essential oils in bioactive edible coatings. Food Eng Rev 3:1–16

Schulz M, Kussmann P, Knop M, Kriegs B, Gresens F, Eichert T, Ulbrich A, Marx F, Fabricius H, Goldbach H, Noga G (2007) Allelopathic monoterpenes interfere with *Arabidopsis thaliana* cuticular waxes and enhance transpiration. Plant Signal Behav 2(4):231–239

Soliman KM, Badeaa RI (2002) Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. Food Chem Toxicol 40:1669–1675

Soylu EM, Kurt S, Soylu S (2010) In vitro and in vivo antifungal activities of the essential oils of various plants against tomato grey mould disease agent *Botrytis cinerea*. Int J Food Microbiol 143:183–189

Srivastava B, Singh P, Shukla R, Dubey NK (2008) A novel combination of the essential oils of *Cinnamomum camphora* and *Alpinia galanga* in checking aflatoxin B_1 production by a toxigenic strain of *Aspergillus flavus*. World J Microbiol Biotechnol 24:693–697

Straede A, Heinisch JJ (2007) Functional analyses of the extra- and intracellular domains of the yeast cell wall integrity sensors Mid2 and Wsc1. FEBS Lett 581:4495–4500

Teng Y, Yang QYuZ, Zhou G, Sun Q, Jin H, Hou T (2010) In vitro antimicrobial activity of the leaf essential oil of *Spiraea alpina* Pall. World J Microbiol Biotechnol 26:9–14

Tsuyuki R, Yoshinari T, Sakamoto N, Nagasawa H, Sakuda S (2011) Enhancement of Trichothecene Production in *Fusarium graminearum* by Cobalt Chloride. J Agric Food Chem 59:1760–1766

Vechet L, Burketova L, Sindelarova M (2009) A comparative study of the efficiency of several sources of induced resistance to powdery mildew (*Blumeria graminis* f. sp. *tritici*) in wheat under field conditions. Crop Prot 28:151–154

Velluti A, Sanchis V, Ramos AJ, Marín S (2004) Effect of essential oils of cinnamon, clove, lemongrass, oregano and palmarosa on growth of and fumonisin B_1 production by *Fusarium verticillioides* in maize. J Sci Food Agric 84:1141–1146

Velluti A, Sanchis V, Ramos AJ, Egido J, Marìn S (2003) Inhibitory effect of cinnamon, clove, lemongrass, oregano and palmarose essential oils on growth and fumonisin B_1 production by *Fusarium proliferatum* in maize grain. Int J Food Microbiol 89:145–154

Williams JH, Phillips TD, Jolly PE, Stiles JK, Jolly CM, Aggarwal D (2004) Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. The Am J Clin Nutr 80:1106–1122

Yaguchi A, Yoshinari T, Tsuyuki R, Takahashi H, Nakajima T, Sugita-Konishi Y, Nagasawa H, Sakuda S (2009) Isolation and identification of precocenes and piperitone from essential oils as specific inhibitors of thricothecene production by *Fusarium graminearum*. J Agric Food Chem 57:846–851

Yoshimura H, Sawai Y, Tamotsu S, Sakai A (2011) 1,8-cineole inhibits both proliferation and elongation of BY-2 cultured tobacco cells. J Chem Ecol 37:320–328

Yoshinari T, Yaguchi A, Takahashi-Ando N, Kimura M, Takahashi H, Nakajima T, Konichi Sugita, Nagasawa, Sakuda (2008) Spiroethers of German chamomile inhibit production of aflatoxin G and trichothecene mycotoxin by inhibiting cytochrome P450 monooxygenases involved in their biosynthesis. FEMS Microbiol Lett 284:184–190

Chapter 15
Plant Essential Oils as Antifungal Treatments on the Postharvest of Fruit and Vegetables

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Abstract Food safety is one of the major issues related to fresh fruit and vegetables. Microbial growth is one of the most important causes of postharvest fruit losses, being fungi the main causal agent associated with the postharvest diseases. The preservation of the 'freshness' quality of these products is relevant due to their economical impact. As an alternative to synthetic preservatives, natural antimicrobial agents have attracted the attention of modern consumers and the fresh produce industry. Particularly, natural antimicrobials based on plant essential oils are gaining support. This chapter is a comprehensive review of the use of essential oils from different sources and their constituents on the control of postharvest fungal decay and overall quality preservation of fresh fruit and vegetables. Emphasis has been on the sources of essential oils and their constituents studied up to now, and their effects on controlling postharvest fungal decay, either *in vitro* or *in vivo*, and their effect on overall quality and storage life of fresh commodities.

15.1 Introduction

Fruit and vegetables are important components of the human diet and their consumption is essential in healthy diets, preventing a wide number of chronic diseases (Wiley 2000). Fresh fruits and vegetables are highly perishable products as a

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cause of their intrinsic characteristics. Microbial growth, sensorial attributes decay and loss of nutrients are amongst the major causes that compromise quality and safety of fresh produce (Ayala-Zavala et al. 2008a, b). In many cases, decay of commodities is only apparent at the latest steps of the handling process. This latent damage can be the result of physical impact, stress injury or quiescent infections by fungi started at the preharvest period. Chemical synthetic additives can reduce decay rate, but consumers are concerned about chemical residues in the product, which could affect their health and cause environmental pollution (White and McFadden 2008), thereby giving rise to the need of developing alternative methods for controlling fresh fruit and vegetable decay. A new worldwide trend to explore alternatives that control postharvest diseases, giving priority to decay-preventing methods with a minimal impact on human health and environment (Bautista-Banos et al. 2006) has emerged. Indeed, the recent exploitation of natural products to control biological spoilage and extend the storage life of perishables has received more and more attention (Tripathi and Dubey 2004). Many fungi that can be the cause of food decay can be inhibited using natural compounds (Fisher and Phillips 2008). Among these, several essential oils, alcohols, organic acids and aromatic compounds have resulted to be biologically active against microbial growth. Particularly, natural antimicrobials based on plant essential oils are gaining support (Isman 2000). These natural compounds are generally recognised as safe (GRAS) for environment and human health, so interest in their use in the goal for sustainable agriculture has increased and a lot of research has been done (Ayala-Zavala et al. 2008c) proving, in many cases, that plant-essential oils and extracts have a role as food preservatives (Hammer et al. 2001). The main reason for promoting the application of natural products in fresh fruits and vegetables is the consumer's demand for natural and/or organic methods to preserve foods. There is an increasing portion of consumers choosing convenient and ready-to-use fruits and vegetables with a fresh-like quality, containing only natural ingredients (Roller and Lusengo 1997). In addition, consumers have become more health-conscious regarding safety aspects concerning the handling of fruit and vegetables. Therefore, not only the 'external aspect' but also the absence of hazardous substances has become imperious issues for buyers (Drzyzga 2003; Harker et al. 2003; Tripathi and Dubey 2004). Different studies have been focused on improving the efficiency of natural compounds as emerging technologies to preserve fresh fruits safety and quality (Ayala-Zavala et al. 2008a, c, d). However, regulatory actions on the use of natural alternative additives are still being analysed. Demands from increasingly mistrustful consumers have led to numerous legislation reviews, which are expected to result in well-planned laws regarding regulations on natural food additives. The main objective of this chapter is to compile the knowledge concerning the use of essential oils in the postharvest fungal decay control of both fruit and vegetables, their efficiency and safety, and to address critical issues requiring further study. In approaching the subject, related issues, such as food quality and antioxidant properties of essential oils were included in this review.

15.2 Fungal Decay as a Major Problem During the Postharvest of Fruit and Vegetables

Harvested fresh produce exhibits two lines of defence: a physical (skin or peel) and a chemical barrier (proteins, cell walls modifications, organic acids, phenols and phytoalexins) against microbial growth (Uritani 1999; Wisniewski et al. 2003). Only a small fraction of bacterial and fungal decay agents can enter the tissue either by their natural openings (e.g. stomata, lenticels) or by direct penetration of the intact cuticle (Wiley 2000). Natural fresh produce defences against microorganisms can be weakened and compromised by stress injury and/or physiological disorders arising from either preharvest and/or handling factors. This is because injuries and disorders cause disruption of tissues that compromises the integrity of commodities, providing favourable conditions for the invasion of decay agents (Batu 2003). Postharvest processing operations include unitary units such as peeling, cutting, shredding or slicing greatly increase tissue damage of fresh-cut fruits. These may result in several biochemical deteriorations such as browning, flavours, loss of texture as well as detriment of nutritional value and microbial quality of the products. Increases in microbial populations on minimally processed products are associated with damaged tissues and broken cells, as microbial growth is much greater on fresh-cut products than intact product. Cell disruption leads to the release and intermixing of enzymes and substrates that may be used by native or exogenous microorganisms to grow on the product.

Fungi, the collective term for a rather large group of related eukaryotic organisms, are the most important group of postharvest decay causal agents, leading to loss of quality of fresh commodities and economic loss in the postharvest period. Moulds are probably the most well known and have the greatest impact on the postharvest quality of fruit and vegetables. Their growth in food crops are also responsible for off-flavour formation which lead to quality losses (Nielsen and Rios 2000). Fungi, besides being responsible for biological spoilage of fresh produce, can also cause foodborne illnesses. In fact, many fungi responsible for the decay of horticultural commodities, besides breaking the natural barriers against other microorganisms, such as bacteria and other human pathogens, can also produce toxic metabolites in the affected sites, named mycotoxins (Tournas 2005). These highly toxic compounds produced by fungi in the genera *Aspergillus*, *Penicillium*, *Alternaria* and *Fusarium* are mainly present in pome and stone fruit, and are often carcinogenic and teratogenic. The severity of the toxicity depends on the type of mycotoxin in question, the respective dose and the person consuming it (Tournas 2005).

In general, for each species of fruit or vegetables, it is possible to find a wide range of diseases caused by different agents. Regarding diseases in fruit and vegetables caused by fungi, these are mostly microscopic and include species from all classes: lower fungi (or Phycomycetes), Ascomycetes, Basidiomycetes and Deuteromycetes (Kushalappa and Zulfiqar 2001). Spores and vegetative cells of yeasts and moulds are abundant in the atmosphere and on the surface of fruits and

vegetables as they approach maturity in the field. In general, fungi are very sensitive to low temperature and most of them are not able to penetrate the surface of the host. However, once they gain entry through wounds or natural openings, they may cause extensive rotting of mature produce (Dennis 1987). Indeed, they are capable of secreting pectic enzymes that cause the maceration of tissues, producing highly severe soft rots (Kushalappa and Zulfiqar 2001).

It is important to note, that all interactions between the commodity and the respective pathogenic agent are largely influenced by environmental factors, notably temperature, pH, water activity, nutrients availability, atmosphere composition, competition imposed by other organisms and presence of antimicrobial compounds (Kushalappa and Zulfiqar 2001). In particular, concerning abiotic factors, there is an optimum level for each specific microorganism at which it can find the appropriate conditions to grow and reproduce extensively. At these optimal conditions, decay agents cause great infections and have a large impact on the food quality. One should also have in mind that the intrinsic conditions of commodities also change widely during ripening, with the decay microflora changing accordingly, starting with fungal infections and followed by bacterial ones, or vice versa. For instance, horticultural produce pH is a major factor in the prevalence of fungal caused diseases in fruit, due to their higher tolerance to acidic environments. On the other hand, vegetables are more susceptible to bacteria, due to their higher pH. Furthermore, moulds are able to neutralise mildly acidic foods, which can lead to safety problems because acidity is often relied on to prevent the growth of decay or pathogenic bacteria (Wade and Beuchat 2003).

Several postharvest treatments have been developed to preserve the quality of fresh produce, including ultraviolet light, controlled and modified atmospheres, edible coatings, heat treatments and natural compounds, among others. Most postharvest treatments involve the alteration of the natural conditions of the fruit in order to prolong its postharvest life. For example, high O_2 atmospheres and irradiation cause damage to some vital molecules of food deteriorative microorganisms, in addition to altering some biochemical processes in the fruit (Charles et al. 2009); heat treatments affect a wide range of fruit ripening processes such as ethylene synthesis, respiration, softening and cell-wall metabolism (Zhang et al. 2009). Gas composition in storage atmospheres might also be more or less favourable to disease spreading, either by fungi or bacteria, although a general trend is not possible to define (Jacques and Morris 1995; DeEll et al. 2003; Gomez and Artes 2004).

To manage postharvest losses caused by fungi, producers usually rely on a release of chemical fungicides (group of benzimidazoles, aromatic hydrocarbons). Currently, there is a strong debate about the safety aspects of chemical preservatives since they are considered responsible for many carcinogenic and teratogenic attributes as well as residual toxicity. For these reasons, consumers tend to be suspicious of chemical additives and thus the demand for natural preservatives has been intensified. The use of synthetic chemicals to control postharvest decay has been restricted to few fruit and vegetables, due to their high and acute residual toxicity, long degradation period, environmental pollution, effects on food and

other side-effects on humans (Lingk 1991; Mari and Guizzardi 1998; Tripathi and Dubey 2004).The increase of fungal resistance to classical drugs, the treatment costs and the fact that most available antifungal drugs have only fungistatic activity, justify the search for new strategies (Rapp 2004). Altogether, this has stimulated intensive research efforts to find alternatives to synthetic chemicals, such as new technologies, substances and practices to be used in postharvest preservation (Jobling 2000; Terry and Joyce 2004; Tripathi and Dubey 2004; Bokshi et al. 2007). Thus, the replacement of synthetic fungicides by natural products (particularly of plant origin), which are non-toxic and specific in their action, is gaining considerable attention (Tripathi and Dubey 2004; Bautista-Banos et al. 2006; Bajpai et al. 2008).

15.3 Essential Oils with Antifungal Power

Natural antimicrobial compounds are a re-emerging alternative to fresh produce preservation (Corbo et al. 2009). The antimicrobial power of plants and herb extracts has been recognised for centuries, and mainly used as natural medicine. Plant volatiles have been widely used as food flavouring agents, and many are generally GRAS. Essential oils (EOs), also called volatile oils, are aromatic oily liquids obtained from plant materials (flowers, herbs, buds, leaves, fruits, twigs, bark, seeds, wood and roots). EOs can be obtained by extraction, fermentation or expression, but steam distillation is the most commonly used method.

Essential oils are very complex natural mixtures which contain about 20–60 components at quite different concentrations. They are characterised by two or three major components at fairly high concentrations (20–70 %) compared to others components present in trace amounts. For example, carvacrol (30 %) and thymol (27 %) are the major components of the *Origanum compactum* essential oil, linalol (68 %) of the *Coriandrum sativum* essential oil, α- and *β*-thuyone (57 %) and camphor (24 %) of the *Artemisia herba-alba* essential oil, 1,8-cineole (50 %) of the *Cinnamomum camphora* essential oil, α-phellandrene (36 %) and limonene (31 %) of leaf and carvone (58 %) and limonene (37 %) of seed *Anethum graveolens* essential oil, menthol (59 %) and menthone (19 %) of *Mentha piperita* essential oil. Generally, these major components determine the biological properties of the essential oils. The components include different groups of distinct biosynthetical origin depending on the plant source (Croteau et al. 2000; Betts 2001; Bowles 2003; Pichersky et al. 2006). The main group is composed of terpenes and terpenoids and the other of aromatic and aliphatic constituents, all characterised by low molecular weight.

The inherent aroma and antimicrobial activity of EOs are related commonly to the chemical configuration of the components, to the proportions in which they are present and to interactions between them, affecting their bioactive properties (Fisher and Phillips 2008). Considering the complex mixture of EOs constituents is difficult to attribute the antimicrobial mode of action to one specific mechanism,

being reported several targets in the microbial cell. It seems that they may cause deterioration of cell wall, damage to cytoplasmic membrane, damage to membrane proteins, leakage of cell contents, coagulation of cytoplasm, depletion of proton motive active sites, inactivation of essential enzymes and disturbance of genetic material functionality (Burt 2004; Ayala-Zavala et al. 2008b; Gutierrez et al. 2008).

Several EOs such as oils of garlic, cinnamon, thyme, oregano, clove, basil, coriander, citrus peel, laurel, ginger, rosemary and peppermint, among others, have been studied as antimicrobial natural products against both bacteria and moulds (Burt 2004; Burt et al. 2005; Ayala-Zavala et al. 2008c, d; Corbo et al. 2009). The biological activity of essential oils and/or their constituents can act as fungistatic and/or fungicidal agents, this depending, for instance, on the concentrations used. Indeed, cinnamon (*Cinnamomum zeylanicum* L.) and clove (*Syzygium aromaticum* L.), essential oils tested against anthracnose (*Colletotrichum musae*) and crown rot pathogens (*Lasiodiplodia theobromae*, *C. musae* and *Fusarium proliferatum*) isolated from banana, showed, *in vitro*, fungistatic and fungicidal activity against these decay agents within the range 0.3–1.1 mg/L. (Jobling 2000; Ranasinghe et al. 2002) The essential oil components carvone, cuminaldehyde, perillaldehyde, cinnamaldehyde, salicylaldehyde and benzaldehyde were found to be the most potent inhibitors of *in vitro* growth of *Penicillium hirsutum* (Smid et al. 1995). Fungal growth inhibition by carvone was found to be reversible, but exposure to cuminaldehyde, perillaldehyde, cinnamaldehyde and salicylaldehyde caused irreversible inhibition of fungal growth. Specifically, cinnamaldehyde has been shown to be a very potent fungicidal agent. Smid et al. (1995) found a 40-fold reduction of the fungal population when dipping tulip bulbs in an aqueous solution of 515 mg/L cinnamaldehyde.

The same essential oil and/or respective compounds can be active against a wide spectrum of microorganism species, although the minimum inhibitory concentration (MIC) used can be very changeable, according to the microbial species and/or the commodity. A varying degree of growth inhibition by the essential oils of several plants against some decay agents, such as *Fusarium*, *Botrytis* and *Aspergillus* spp., due to their different chemical composition, has been reported (Singh et al. 2002; Bouchra et al. 2003). Bajpai et al. (2008) found that the essential oil isolated from the floral parts of *Silene armenia* L. had a remarkable antifungal effect, against not only *B. cinerea* growth *in vitro* but also other critical decay agents. Cassia oil completely inhibited the *in vitro* growth of *Alternaria alternata* at 300 or 500 mg/L exposure for 6 and 3 days, respectively. When applied to tomatoes, cassia oil at 500 mg/L reduced the percentage of decay by 40–50 % (Feng and Zheng 2007).

Interestingly, the antifungal kinetics of *S. armenia* essential oil tested against *B. cinerea* correlated positively with increased exposure time and oil concentration. At concentrations of 62.5 and 125 mg/L, the fungicidal activity was very rapid (120 and 150 min, respectively), due to the presence of 2-butene, caryophyllene oxide, methylcyclopropane and α-butylene components in the oil (Bajpai et al. 2008).

However, the fungus *Sclerotinia sclerotiorum* was found to be slightly resistant to the same oil, showing a MIC of 1000 mg/L.

Otherwise, different essential oils and/or compounds obtained from different plant species can exhibit different MICs for the same microbial agent. Bouchra et al. (2003) found that the essential oils of *Origanum compactum* Benth and *Thymus glandulosus* Req., consisting mainly of carvacrol and thymol, were the most efficient in the control of *B. cinerea*, by completely inhibiting mycelial growth *in vitro* at 100 mg/L. In contrast, essential oils of species such as *Chenopodium ambrosioides* L., *Eucalyptus citriodora* Hook, *Eupatorium cannabinum* L., *Lawsonia inermis* L., *Ocimum canum* Sim., *Ocimum Gratissimum* L., *Ocimum Sanctum* L., *Prunus persica* (L.) Batsch, *Zingiber cassumunar* Roxb and *Zingiber officinale* Rosc were found to exhibit *in vitro* fungitoxic activity against *B. cinerea* at 500 mg/L (Tripathi et al. 2008). When used to control grey mould in grapes caused by *B. cinerea* during storage, essential oils from *O. sanctum*, *P. persica* and *Z. officinale* showed MIC values of 200, 100 and 100 mg/L, respectively, and promoted the enhancement of storage life up to 4–6 days (Tripathi et al. 2008). Thyme oil exhibited a higher degree of inhibition of *A. alternata* (62.0 % at 500 mg/l) than cassia oil (40–50 % at 500 mg/l) in tomato (Feng and Zheng 2007).

The mechanism underlying the action of essential oil enrichment on the switch between vegetative and reproductive phases of fungal development remains to be fully understood. The negative impact of essential oils on fungi sporulation may reflect the effect of the volatiles emitted by oils on surface mycelia development and/or the perception/transduction of signals involved in the switch from vegetative to reproductive development (Tzortzakis 2007).

Nevertheless, suppression of spore production by essential oils could play a major role in limiting the spread of the pathogen, by lowering the spore load in the storage atmosphere and on surfaces (Tzortzakis 2007). Tzortzakis and Economakis (2007) found that in *Colletotrichum coccodes*, *Botrytis cinerea*, *Cladosporium herbarum* and *Rhizopus stolonifer*, spore production grown *in vitro* was reduced up to 70 % when exposed to 25 mg/L lemongrass (*Cympopogon citratus* L.) essential oil, and completely inhibited at 500 mg/L. In contrast, the same authors found that lemongrass oil (up to 100 mg/L) accelerated spore germination in *Aspergillus niger*. This, however, was reversed at 500 mg/L, when the process was fully inhibited, as for the other pathogens, due to failure of spore production.

The application mode of essential oils/their compounds has shown to have different results. For instance, spraying with basil oil (*Ocimum basilicum* L.) emulsion of 160 mg/L controlled crown rot and anthracnose, prolonging the storage life of 'Embul' bananas (Anthony et al. 2003). Eucalyptus (*Eucaliptus globules* L.) and cinnamon (*Cinnamomum zeylanicum*, Blume) are essential oil vapours applied at 50 mg/L concentrations for 8 h at 20 °C reduced fruit decay and improved the quality of tomatoes and strawberries during late storage life (Tzortzakis 2007). It is noteworthy that, when applied at 500 mg/L, the results obtained for the same compounds were similar. Tsao and Zhou (2000) found that thymol and carvacrol were effective in controlling brown rot caused by *Monilinia fructicola* in sweet cherries (*Prumus avium* L.), in either dipping or fumigation.

B. cinerea and *Alternaria arborescens*, isolated from tomatoes, showed complete *in vitro* growth inhibition when exposed to oregano (*Oreganum vulgare* L.), thyme (*Thymus vulgaris* L.) and lemongrass (*Cymbopogon citrates* L.) vapours at 50 mg/L for up to 12 h (Plotto et al. 2003). *Geotrichum candidum* was more sensitive to lemongrass oil (citral) vapours than to thyme or oregano oils. The same authors reported that only vapours of thyme and oregano oils (thymol and carvacrol) inhibited *R. stolonifer*. Interestingly, when incorporated into the growth medium, thyme and oregano oils showed a fungicidal or fungistatic activity for all four fungi at 500 mg/L, while lemongrass oil has the same effect at only 1,000 mg/L for all species except *Rhizopus*, for which no inhibition was reported. Cilantro (*Coriandrum sativum* L.) oil (*trans*-2-decenal) was fungicidal to *Botrytis*, *Alternaria* and *Geotrichum* as vapour, but lost its activity when incorporated into the growth medium (Plotto et al. 2003). However, none of the essential oils used by Plotto et al. (2003) *in vitro* succeeded in controlling disease development in tomato fruits inoculated with *B. cinerea*, *A. arborescens* or *R. stolonifer*, when applied as vapours. Moreover, phytotoxicity has been observed in fruits after 24 h exposure, either in tomatoes fumigated with the essential oils or with their respective major constituents alone. When dip treatments were done at 5,000 and 10,000 mg/L, it was found that thyme and oregano oils reduced disease development in tomatoes inoculated with *B. cinerea* or *A. arborescens* but also caused some phytotoxicity at those concentrations if the emulsion was not complete (Plotto et al. 2003). Lemongrass was more phytotoxic at 10,000 mg/L than thyme or oregano oils, probably due to some compounds present in this oil. Neither thyme nor oregano oils could control *Rhizopus* spp. inoculated in tomato wounds. On the contrary, the higher the concentration applied, the more the disease developed, probably due to a local phytotoxic effect of the essential oils in the wound, making the tissue more susceptible to this pathogen (Plotto et al. 2003).

Methyl jasmonate (MJ) is a natural compound widely distributed in plants. It was first detected as a sweet fragrant compound in *Jasminum* essential oil and other plant species (González-Aguilar et al. 2006). MJ is known to regulate plant development and response to environmental stress (Demo et al. 2005; Yao and Tian 2005), affecting many biochemical and physiological reactions in the tissue of whole and fresh-cut fruits and vegetables and extending shelf-life of whole and fresh-cut tomatoes, mangoes, guavas and strawberries (González-Aguilar et al. 2006). Ayala-Zavala et al. (2005, 2008c) reported that MJ alone or in conjunction with ethanol treatment increased antioxidant capacity, volatile compounds and post-harvest life of strawberry fruit, as well as extending shelf-life of fresh-cut tomatoes, suppressing fungal growth. Methyl jasmonate (MJ) either as a vapour or as an emulsion has shown to suppress green mould growth on grapefruit (Droby et al. 1999) and inhibit grey mould infection on strawberries alone or as a co-fumigant with ethanol (Ayala-Zavala et al. 2005).

15.3.1 Oils Rich in Terpenes

Terpenes form structurally and functionally different classes. The main terpenes are the monoterpenes (C10) and sesquiterpenes (C15), but hemiterpenes (C5), diterpenes (C20), triterpenes (C30) and tetraterpenes (C40) also exist. A terpene containing oxygen is called a terpenoid. The monoterpenes are formed from the coupling of two isoprene units (C10). They are the most representative molecules constituting 90 % of the essential oils and allow a great variety of structures. They consist of several functions, which are displayed in Table 15.1.

The sesquiterpenes are formed from the assembly of three isoprene units (C15). The extension of the chain increases the number of cyclisations which allows a great variety of structures. The structure and function of the sesquiterpenes are similar to those of the monoterpenes.

Examples of plants containing these compounds are angelica, bergamot, caraway, celery, citronella, coriander, eucalyptus, geranium, juniper, lavandin, lavander, lemon, lemongrass, mandarin, mint, orange, peppermint, petitgrain, pine, rosemary, sage, thyme.

15.3.2 Oils Rich in Phenolic Compounds

Derived from phenylpropane, the phenolic compounds occur less frequently than the terpenes. The biosynthetic pathways concerning terpenes and phenylpropanic derivatives generally are separated in plants but may coexist in some, with one major pathway taking over (see, cinnamon oil with cinnamaldehyde as major and eugenol as minor constituents, also clove oil, fennel, etc.). The phenolic compounds are depicted in Table 15.1.

The phenolic compounds found in essential oils normally have a carbon side chain and here we can look at compounds such as thymol, eugenol and carvacrol, that are classified as monoterpenic phenolic compounds. These components have great antiseptic, anti-bacterial and disinfectant qualities and also have greatly stimulating therapeutic properties. Evidence suggests that phenol induces progressive loss of intracellular constituents from treated bacteria and produces generalised membrane damage with intracellular coagulation occurring at higher concentrations. The plasma membrane of fungi is also damaged. The mechanisms thought to be responsible for the phenolic toxicity to microorganisms include enzyme inhibition by the oxidised compounds, possibly through reaction with sulphydryl groups or through more non-specific interactions with the proteins. Phenols are always present in conjugated form, usually with glucosidic attachment. They may be released in the free form during the fungal infection through enzymatic or other hydrolysis mechanisms. The site(s) and number of hydroxyl groups on the phenol group are thought to be related with the relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased

Table 15.1 Different active compounds found in essential oils

Terpenes		Phenolic compounds	Sulphur riched compounds
Monoterpenes	Sesquiterpenes		
Carbures:	*Carbures*:	*Aldehyde*: cinnamaldehyde	Principal components are diallyl monosulphide, diallyl disulphide, diallyl trisulphide and diallyl tetrasulphide
Acyclic: myrcene, ocimene	Azulene, b-bisabolene, cadinenes,	*Alcohol*: cinnamic alcohol	
Monocyclic: terpinenes, p-Cimene, phellandrenes	B-caryophyllene, logifolene, curcumenes, elemenes, farnesenes, zingiberene	*Phenols*: chavicol, eugenol,	
Bicyclic: pinenes, -3-carene, camphene, sabinene	*Alcohols*: bisabol, cedrol, b-nerolidol, farnesol, carotol, b-santalol, patchoulol, viridiflorol	Thymol, carvacrol	
Alcohols:	*Ketones*: germacrone, nootkatone, cis-longipinan-2,7- dione, b-vetinone, turmerones	*Methoxy derivatives*: anethole, elemicine, estragole, methyleugenols	
Acyclic: geraniol, linalol, citronellol, lavandulol, nerol	*Epoxide*: caryophyllene oxide, humulene epoxides	*Methylene dioxy compounds*: apiole, myristicine, safrole	
Monocyclic: menthol, a-terpineol, carveol bicyclic: borneol, fenchol, chrysanthenol, thuyan-3-ol			

(continued)

Table 15.1 (continued)

Terpenes		Phenolic compounds	Sulphur riched compounds
Monoterpenes	Sesquiterpenes		
Aldehydes:			
Acyclic: geranial, neral, citronellal			
Ketone:			
Acyclic: tegetone			
Monocyclic: menthones, carvone, pulegone, piperitone			
Bicyclic: camphor, fenchone, thuyone, ombellulone, pinocamphone, pinocarvone			
Esters:			
Acyclic: linalyl acetate or propionate, citronellyl acetate			
Monocyclic: menthyl or a-terpinyl acetate			
Bicyclic: isobornyl acetate			
Ethers:			
1,8-cineole, menthofurane			
Peroxydes: ascaridole			
Phenols: thymol, carvacrol			

toxicity (Troncoso-Rojas and Tiznado-Hernández 2007). Due to the nature of phenols, essential oils that are high in them should be used in low concentrations and for short periods of time, since they can lead to toxicity if used over long periods of time. The principal plant sources for these compounds are anise, cinnamon, clove, fennel, nutmeg, parsley, sassafras, star anise, tarragon and some botanical families (Apiaceae, Lamiaceae, Myrtaceae, Rutaceae).

15.4 Sulphur-Riched Compounds

These compounds are another kind of natural volatiles that had been shown a strong antifungal activity. They are present in several plants like onion, garlic and others. The active constituents of garlic and onion are sulphur-riched compounds that are rapidly absorbed and metabolised. Allicin is considered to be the most important biologically active compound in garlic; however, during processing of garlic this compound is transformed to other sulfur compounds. Chemical analysis of garlic showed that 54.5 % of the total sulphides were the sum of diallyl monosulphide, diallyl disulphide, diallyl trisulphide and diallyl tetrasulphide (Troncoso-Rojas and Tiznado-Hernández 2007). Most of the reports in the literature regarding the antimicrobial effect of garlic oil are referent to antibacterial activity; while a little information about the antifungal activity is reported.

15.5 Collateral Effects of the Use of Essential Oils as Treatment of Fruit and Vegetables

The postharvest antifungal activity of essential oils is well and positively documented from a wide number of *in vitro* and *in vivo* experiments. The concentrations of essential oils and the respective compounds necessary to inhibit microbial growth are usually higher in foods than in culture media, which might be the result of interactions between EOs compounds and the food matrix (Nuchas and Tassou 2000) and should be taken into account in commercial applications (Tzortzakis 2007). Normally, direct application of antimicrobials to food must be done at high concentrations to achieve good antimicrobial activity against target microorganisms in food produce meant to be stored for an extended period of time. The ways in which EOs are applied and the concentrations at which they are used are important factors related to their effectiveness, and in some circumstances. EOs could be the cause of changes in flavour, odour and other characteristics of food products due to the strong odour-flavour that can be transmitted from the oil to the vegetable product. The chemical reactivity of EOs with the food and package matrix could significantly affect the sensorial properties of the produce (Ayala-Zavala et al. 2008d). The intense sensory attributes of some of these compounds

may also be an impediment for their use in fresh commodities and therefore, in the application of natural antimicrobial and flavouring compounds such as fresh-cut fruits and vegetables preservatives, the sensorial impact should be considered (Ayala-Zavala et al. 2008a). A residual taste from thymol on fumigated cherries made this alternative treatment not commercially applicable. However, the same authors have found that when tomatoes were treated at the 'breaker' or 'turning' stages, enough time passed from initial application to eating maturity to allow volatilization, since no residual taste appeared on the tomatoes 10 days after treatment. Encapsulation in β-cyclodextrin (β-CD) is one method to control the odour and reactivity of active compounds throughout the release of natural antimicrobial compounds (Ayala-Zavala et al. 2008d). Microencapsulation can be a solution to solve this problem, because during the microencapsulation process, the active antimicrobial compounds will be trapped, masking odour and flavour until release to the atmosphere in constant low doses (Del Toro-Sánchez et al. 2010). This can protect the product from microbial growth without affecting its sensory acceptability.

Essential oils, as natural sources of phenolic components, attract investigators to evaluate their activity as antioxidants or free radical scavengers. The essential oils of basil, cinnamon, clove, nutmeg, oregano and thyme have proven radical-scavenging and antioxidant properties in the DPPH radical assay at room temperature (Tomaino et al. 2005). The order of effectiveness was found to be: clove $\gg$ cinnamon $>$ nutmeg $>$ basil $\geq$ oregano $\gg$ thyme. The essential oil of *Thymus serpyllum* showed a free radical scavenging activity close to that of the synthetic butylated hydroxytoluene (BHT) in a β-carotene/linoleic acid system (Tepe et al. 2005). The antioxidant activity was attributed to the high content of the phenolics thymol and carvacrol (20.5 and 58.1 %, respectively). *Thymus spathulifolius* essential oil also possessed an antioxidant activity due to the high thymol and carvacrol content (36.5, 29.8 %, respectively; Sokmen et al. 2004). The antioxidant activity of oregano (*Origanum vulgare* L., ssp. hirtum) essential oil was comparable to that of α-tocopherol and BHT, but less effective than ascorbic acid (Kulisic et al. 2004). The activity is again attributed to the content of thymol and carvacrol (35.0, 32.0 %, respectively).

The essential oils of *Salvia cryptantha* and *Salvia multicaulis* have the capacity to scavenge free radicals. The activity of these oils was higher than that of curcumin, ascorbic acid or BHT (Tepe et al. 2004). In addition, *Curcuma zedoaria* essential oil was found to be an excellent scavenger for DPPH radical (Mau et al. 2003).The antioxidant activity of essential oils cannot be attributed only to the presence of phenolic constituents; monoterpene alcohols, ketones, aldehydes, hydrocarbons and ethers also contribute to the free radical scavenging activity of some essential oils. For instance, the essential oil of *Thymus caespititius*, *Thymus camphorates* and *Thymus mastichina* showed antioxidant activity which in some cases was equal to that of α-tocopherol (Miguel et al. 2004). Surprisingly, the three species are characterised by high contents of linalool and 1,8-cineole, while thymol or carvacrol are almost absent. The essential oil of lemon balm (*Melissa officinalis* L.) shows an antioxidant and free radical scavenging activity (Mimica-Dukic et al. 2004) with

the most powerful scavenging constituents comprising neral/geranial, citronellal, isomenthone and menthone. Tea tree (*Melaleuca alternifolia*) oil has been suggested as a natural antioxidant alternative for BHT (Kim et al. 2004) with the inherent antioxidant activity attributed mainly to the α-terpinene, γ-terpinene and α-terpinolene content. Essential oils isolated from *Mentha aquatica* L., *Mentha longifolia* L. and *Mentha piperita* L., were able to reduce DPPH radicals into the neutral DPPH-H form (Mimica-Dukic et al. 2003). The most powerful scavenging constituents were found to be 1,8-cineole for the oil of *M. aquatica* while menthone and isomenthone were the active principles of *M. longifolia* and *M. piperita.* It is clear that essential oils may be considered as potential natural antioxidants and could perhaps be formulated as a part of daily supplements or additives to prevent oxidative stress that contributes too many degenerative diseases. And its addition to fruit and vegetables can cause the increment of the antioxidant activity of the treated produce.

15.6 Physiological Effects

Many authors mentioned beneficial or no detrimental effects on horticultural product quality parameters when essential oils are used after harvest. Tzortzakis (2007) reported that decay was reduced in strawberries and tomatoes by the use of essential oils from eucalyptus and cinnamon, with no effects on fruit firmness. Similar results were observed for cherries and grapes when treated with vapours of eugenol, thymol or menthol (Martinez-Romero et al. 2005). Chinese pears (*Pyrus bertschneideri* Reld cvs. Laiyang Chili and Ya Li) treated with emulsions (3–9 %) of commercial or refined (reduced a-tocopherol) plant oils (soybean, corn, olive, peanut, linseed and cottonseed) at harvest and stored for 6 months at 0 °C maintained firmness in a concentration-dependent manner during storage (Ju et al. 2000). In the same way, quality attributes such as colour, soluble solids content and titratable acidity were preserved through storage. Pears showed no off-flavours when compared to controls and internal ethanol was not affected by oil treatment. The higher concentrations reduced internal browning of Chinese pears and scald incidence in 'Delicious' apples (Ju et al. 2000). Oil vapours increased the levels of total soluble solids during exposure in strawberries and tomatoes but the effect persisted following exposure only in 'cherry' tomatoes (Tzortzakis 2007). The same authors reported that fruit samples treated with oil vapours did not differ in percentage weight loss, organic acid content, sweetness and total phenolic content during or following vapour exposure, compared with untreated fruit. Yet table grapes impregnated with 0.5 mL thymol or menthol showed significantly lower weight loss and soluble solids/titratable acidity ratio than controls, as well as reduced firmness and colour changes during storage (Martinez-Romero et al. 2005).

Tsao and Zhou (2000) found that thymol and carvacrol were effective in controlling brown rot caused by the *Monilinia fructicola* in sweet cherries (*Prumus avium* L.), but caused stem browning of cherry fruits in the fumigation experiment.

This side-effect was reduced by 69 and 73 %, respectively, when methyl jasmonate was used as a co-fumigant.

15.7 Conclusion

In the last years many studies have been carried out concerning the antifungal activity of EOs. As this review reveals many EOs possess strong antifungal activity, however, some collateral responses in the treated fresh produce should be evaluated. In the application of natural antimicrobial and flavouring compounds, the sensorial impact should be considered. Therefore, sensorial impact, limited stability and high volatility represent drawbacks of EOs which complicate the *in vitro* tests as well as the storage and application. In addition, the effect of the treatments on the antioxidant and health-related benefits of the treated fruit and vegetables must be contemplated considering the bioactive properties of EOs. Therefore, more research has to be done to develop formulations that maintain the fungicidal activity while not inducing undesirable effects.

References

Anthony S, Abeywickrama K, Wijeratnam SW (2003) The effect of spraying essential oils *Cymbopogon nardus*, *Cymbopogon flexuosus* and *Ocimum basilicum* on postharvest diseases and storage life of Embul banana. J Hortic Sci Biotechnol 78:780–785

Ayala-Zavala JF, Wang SY, Wang CY, Gonzalez-Aguilar GA (2005) Methyl jasmonate in conjunction with ethanol treatment increases antioxidant capacity, volatile compounds and postharvest life of strawberry fruit. Eur Food Res Technol 221:731–738

Ayala-Zavala JF, del Toro-Sánchez L, Alvarez-Parrilla E, Soto-Valdez H, Martín-Belloso O, Ruiz-Cruz S, González-Aguilar GA (2008a) Natural antimicrobial agents incorporated in active packaging to preserve the quality of fresh fruits and vegetables. Stewart Postharvest Rev 4:1–9

Ayala-Zavala JF, Del-Toro-Sánchez L, Alvarez-Parrilla E, González-Aguilar GA (2008b) High relative humidity in-package of fresh-cut fruits and vegetables: advantage or disadvantage considering microbiological problems and antimicrobial delivering systems? J Food Sci 73:R41–R47

Ayala-Zavala JF, Oms-Oliu G, Odriozola-Serrano I, Gonzalez-Aguilar GA, Alvarez-Parrilla E, Martin-Belloso O (2008c) Bio-preservation of fresh-cut tomatoes using natural antimicrobials. Eur Food Res Technol 226:1047–1055

Ayala-Zavala JF, Soto-Valdez H, González-León A, Alvarez-Parrilla E, Martín-Belloso O, González-Aguilar GA (2008d) Microencapsulation of cinnamon leaf (*Cinnamomum zeylanicum*) and garlic (*Allium sativum*) oils in betacyclodextrin. J Incl Phenom Macrocycl Chem 60:359–368

Bajpai VK, Shukla S, Kang SC (2008) Chemical composition and antifungal activity of essential oil and various extract of *Silene armeria* L. Bioresource Technol 99(18):8903–8908

Batu A (2003) Temperature effects on fruit quality of mature green tomatoes during controlled atmosphere storage. Int J Food Sci Nutr 54:201–208

Bautista-Banos S, Hernandez-Lauzardo AN, Velázquez-del Valle MG, Hernandez-Lopez M, Ait-Barka E, Bosquez-Molina E, Wilson CL (2006) Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities. Crop Prot 25:108–118

Betts TJ (2001) Chemical characterization of the different types of volatile oil constituents by various solute retention ratios with the use of conventional and novel commercial gas chromatographic stationary phases. J Chromatogr A 936:33–46

Bokshi AI, Morris SC, McConchie R (2007) Environmentally-safe control of postharvest diseases of melons (*Cucumis melo*) by integrating heat treatment, safe chemicals and systemic acquired resistance. N Z J Crop Hort 35:179–186

Bouchra C, Achouri M, Hassani LMI, Hmamouchi M (2003) Chemical composition and antifungal activity of essential oils of seven Moroccan Labiatae against *Botrytis cinerea* Pers. J Ethnopharmacol 89:165–169

Bowles EJ (2003) The Chemistry of Aromatherapeutic Oils. Allen & Unwin, ISBN 174114051X

Burt SA (2004) Essential oils: their antibacterial properties and potential applications in foods—a review. Int J Food Microbiol 94:223–253

Burt SA, Vlielander R, Haagsman HP, Veldhuizen EJA (2005) Increase in activity of essential oil components carvacrol and thymol against *Escherichia coli* O157:H7 by addition of food stabilizers. J Food Prot 68:919–926

Charles MT, Tano K, Asselin A, Arul J (2009) Physiological basis of UV-C induced resistance to Botrytis cinerea in tomato fruit. Constitutive defense enzymes and inducible pathogenesis-related proteins. Postharvest Biol Technol 51:414–424

Corbo MR, Bevilacqua A, Campaniello D, d'Amato D, Speranza B, Sinigaglia M (2009) Prolonging microbial shelf life of foods through the use of natural compounds and nonthermal approaches—a review. Int J Food Sci Technol 44:223–241

Croteau R, Kutchan TM, Lewis NG (2000) Natural products (secondary metabolites). In: Buchanan B, Gruissem W, Jones R (eds) Biochemistry and molecular biology of plants. American Society of Plant Physiologists, Rockville, pp 1250–1318

DeEll JR, Toivonen PMA, Doussineau J, Roger C, Vigneault C (2003) Effect of different methods for application of an antifog shrink film to maintain cauliflower quality during storage. J Food Qual 26:211–218

Del Toro-Sánchez CL, Ayala-Zavala JF, Machi L, Santacruz H, Villegas-Ochoa MA, Alvarez-Parrilla E, González-Aguilar GA (2010) Controlled release of antifungal volatiles of thyme essential oil from b-cyclodextrin capsules. J Incl Phenom Macrocycl Chem 67:431–441

Demo M, de Las M, Olivia M, Lopez ML, Zunino MP, Zygadlo JA (2005) Antimicrobial activity of essential oils obtained from aromatic plants of Argentina. Pharm Biol 43:129–134

Droby S, Porat R, Cohen L, Weiss B, Shapiro B, Philosoph-Hada S, Meir S (1999) Suppressing green mold decay in grapefruit with postharvest jasmonate application. J Am Soc Hort Sci 124:184–188

Drzyzga O (2003) Diphenylamine and derivatives in the environment: a review. Chemosphere 53:809–818

Feng W, Zheng X (2007) Essential oils to control *Alternaria alternata* in vitro and in vivo. Food Control 18:1126–1130

Fisher K, Phillips C (2008) Potential antimicrobial uses of essential oils in food: is citrus the answer? Trends Food Sci Technol 19:156–164

Gomez PA, Artes F (2004) Controlled atmospheres enhance postharvest green celery quality. Postharvest Biol Technol 34:203–209

González-Aguilar GA, Tiznado-Hernandez M, Wang C-Y (2006) Physiological and biochemical responses of horticultural products to methyl jasmonate. Stewart Postharvest Rev 2:1–9

Gutierrez J, Barry-Ryan C, Bourke P (2008) The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. Int J Food Microbiol 124:91–97

Hammer KA, Carson CF, Riley TV (2001) Antimicrobial activity of essential oils and other plant extracts. J Appl Microbiol 86:985–990

Harker FR, Gunson FA, Jaeger SR (2003) The case for fruit quality: an interpretative review of consumer attitudes, and preferences for apples. Postharvest Biol Technol 28:333–347

Isman MB (2000) Plant essential oils for pest and disease management. Crop Prot 19:603–608
Jobling J (2000) Essential oils: a new idea for postharvest disease control. Good Fruit Vegetables Mag 11:50
Ju Z, Duan Y, Ju Z (2000) Plant oil emulsion modifies internal atmosphere, delays fruit ripening, and inhibits internal browning in Chinese pears. Postharvest Biol Technol 20:243–250
Kim H, Chen F, Wu C, Wang X, Chung H, Jin Z (2004) Evaluation of antioxidant activity of Australian tea tree (Melaleuca alternifolia) oil and its components. J Agric Food Chem 52:2849–2854
Kulisic T, Radonic A, Katalinic V, Milos M (2004) Analytical, nutritional and clinical methods use of different methods for testing antioxidative activity of oregano essential oil. Food Chem 85:633–640
Kushalappa AC, Zulfiqar M (2001) Effect of wet incubation time and temperature on infection, and of storage time and temperature on soft lesion expansion in potatoes inoculated with Erwinia Carotovora ssp. Carotovora. Potato Res 44:233–242
Lingk W (1991) Health risk evaluation of pesticide contaminations in drinking water. Gesunde Pflanz 43:21–25
Mari M, Guizzardi M (1998) The postharvest phase: emerging technologies for the control of fungal diseases. Phytoparasitica 26:59–66
Martinez-Romero D, Castillo S, Valverde JM, Guillen F, Valero D, Serrano M (2005) The use of natural aromatic essential oils helps to maintain postharvest quality of 'Crimson' table grapes. Acta Hort 682:1723–1729
Mau J, Laib E, Wang N, Chen C, Chang C, Chyau C (2003) Composition and antioxidant activity of the essential oil from *Curcuma zedoaria*. Food Chem 82:583–591
Miguel G, Simones M, Figueiredo A, Barroso J, Pedro L, Carvalho L (2004) Composition and antioxidant activities of the essential oils of *Thymus caespititius*, *Thymus camphorates* and *Thymus mastichina*. Food Chem 86:183–188
Mimica-Dukic N, Bozin B, Sokovic M, Mihajlovic B, Matavulj M (2003) Antimicrobial and antioxidant activities of three Mentha species essential oils. Planta Med 69:413–419
Mimica-Dukic N, Bozin B, Sokovic M, Simin N (2004) Antimicrobial and antioxidant activities of *Melissa officinalis* L. (Lamiaceae) essential oil. J Agric Food Chem 52:2485–2489
Nielsen PV, Rios R (2000) Inhibition of fungal growth on bread by volatile components from species and herbs, and the possible application in active packaging, with special emphasis on mustard essential oil. Int J Food Microbiol 60:219–229
Nuchas GE, Tassou CC (2000) Traditional preservatives: oils and spices. In: Robinson RK, Batt CA, Patel PD (eds) Encyclopedia of food microbiology. Academic Press, London, pp 1717–1722
Pichersky E, Noel JP, Dudareva N (2006) Biosynthesis of plant volatiles: nature's diversity and ingenuity. Science 311:808–811
Plotto A, Roberts DD, Roberts RG (2003) Evaluation of plant essential oils as natural postharvest disease control of tomato (*Lycopersicon esculentum* L.). Acta Hort 628:737–745
Ranasinghe L, Jayawardena B, Abeywickrama K (2002) Fungicidal activity of essential oils of *Cinnamomum zeylanicum* (L.) and *Syzygium aromaticum* (L.) Merr et L.M. Perry against crown rot and anthracnose pathogens isolated from banana. Lett Appl Microbiol 35:208–211
Rapp RP (2004) Changing strategies for the management of invasive fungal infections. Pharmacotherapy 24:4–28
Roller S, Lusengo J (1997) Developments in natural food preservatives. Agro Food Industry Hi-Tech 8:22–25
Singh G, Singh OP, Maurya S (2002) Chemical and biocidal investigations on essential oils on some Indian *Curcuma* species. Prog Cryst Growth Charact 45:75–81
Smid EJ, Witte Y, Gorris LGM (1995) Secondary plant metabolites as control agents of postharvest *Penicillium* rot on tulip bulbs. Postharvest Biol Technol 6:303–312
Sokmen A, Gulluce M, Akpulat A (2004) The in vitro antimicrobial and antioxidant activities of the essential oils and methanol extracts of endemic *Thymus spathulifolius*. Food Control 15:627–634

Tepe B, Donmez E, Unlub M (2004) Antimicrobial and antioxidative activities of the essential oils and methanol extracts of *Salvia cryptantha* (Montbret et Aucher ex Benth.) and *Salvia multicaulis* (Vahl). Food Chem 84:519–525

Tepe B, Sokmen M, Akpulat A, Daferera D, Polissiou M, Sokmen A (2005) Antioxidative activity of the essential oils of *Thymus sipyleus* subsp. *sipyleus* var. *sipyleus* and *Thymus sipyleus* subsp. *sipyleus* var. *rosulans*. J Food Eng 66:447–454

Terry LA, Joyce DC (2004) Elicitors of induced disease resistance in postharvest horticultural crops: a brief review. Postharvest Biol Technol 32:1–19

Tomaino A, Cimino F, Zimbalatti V (2005) Influence of heating on antioxidant activity and the chemical composition of some spice essential oils. Food Chem 89:549–554

Tournas VH (2005) Spoilage of vegetable crops by bacteria and fungi and related health hazards. Crit Rev Microbiol 31:33–44

Tripathi P, Dubey NK (2004) Exploitation of natural products as an alternative strategy to control post harvest fungal rotting of fruit and vegetables. Postharvest Biol Technol 32:235–245

Tripathi P, Dubey NK, Shukla AK (2008) Use of some essential oils as post-harvest botanical fungicides inthemanagement of greymould of grapes caused by *Botrytis cinerea*. World J Microbiol Biotech 24:39–46

Troncoso-Rojas R and Tiznado-Hernández ME (2007) Natural compounds to control fungal postharvest diseases. In: Troncoso-Rojas R, Tiznado-Hernández M, González-León A (eds) Recent advances in alternative postharvest technologies to control fungal diseases in fruit and vegetables. Research Signpost, Trivandrum-695 023, Kerala, India pp 127–156

Tsao R, Zhou T (2000) Interaction of monoterpenoids, methyl jasmonate, and Ca^{2+} in controlling postharvest brown rot of sweet cherry. HortScience 35:1304–1307

Tzortzakis NG (2007) Maintaining postharvest quality of fresh produce with volatile compounds. Innovative Food Sci Emerg 8:111–116

Tzortzakis NG, Economakis CM (2007) Antifungal activity of lemongrass (*Cympopogon citratus* L.) essential oil against key postharvest pathogens. Innovative Food Sci Emerg 8:253–258

Uritani I (1999) Biochemistry on postharvest metabolism and deterioration of some tropical tuberous crops. Bot Bull Acad Sinica 40:177–183

Wade WN, Beuchat LR (2003) Proteolytic fungi isolated from decayed and damaged tomatoes and implications associated with changes in pericarp pH favorable for survival and growth of foodborne pathogens. J Food Prot 66:911–917

White JML, McFadden JP (2008) Contact allergens in food ingredients and additives: atopy and the hapten-atopy hypothesis. Contact Dermatitis 58:245–246

Wiley RC (2000) Minimally processed refrigerated fruits and vegetables. Chapman & Hall, New York

Wisniewski ME, Basset CL, Artlip TS, Webb RP, Janisiewickz WJ, Norelli JL, Goldway M, Droby S (2003) Characterization of a defensin in bark and fruit tissues of peach and antimicrobial activity of a recombinant defensin in the yeast, *Pichia pastoris*. Physiol Plant 119:563–572

Yao HJ, Tian SP (2005) Effects of biocontrol agent and methyl jasmonate on postharvest diseases of peach fruit and the possible mechanisms involved. J Appl Microbiol 98:941–950

Zhang Z, Nakano K, Maezawa S (2009) Comparison of the antioxidant enzymes of broccoli after cold or heat shock treatment at different storage temperatures. Postharvest Biol Technol 54:101–105

Chapter 16
Fruit Processing Byproducts as a Source of Natural Antifungal Compounds

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Abstract Nowadays, there has been an increasing concern of consumers on foods free or with lower levels of synthetic chemical preservatives, because they could be toxic for humans and the environment. Concomitantly, consumers have also demanded foods with long shelf life and fruit producers and processors must deal with the perishable character of its products and the large percentage of byproducts, such as peels, seeds, and unused flesh that are generated by different steps of the industrial process. It has been reported that the wasted byproducts present high contents of antifungal compounds, providing a potential alternative to protect foods or feeds from fungal contamination. The aim of this chapter is to highlight the importance of the integral exploitation of the fruit byproducts, analyzing the current state of the situation. Additionally, the chapter reviews the most recent investigations on bioactive compounds with antifungal properties extracted from fruit residuals and their possible utilization as antimicrobials not only for the food but also for the cosmetic and pharmaceutical industries.

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16.1 Introduction

The presence and growth of fungi in food may cause spoilage and result in a reduction in quality and quantity. So far, many molds, such as *Fusarium* spp., *Aspergillus* spp., *Penicillium* spp., and *Rhizopus* spp., have been reported as the causal agents of foodborne diseases and/or food spoilage (Betts et al. 1999). Chemical synthetic additives can reduce food decay, but consumers are concerned about chemical residues in the products (White and McFadden 2008; Ayala-Zavala and González-Aguilar 2011), combined with the assumption that a number of common synthetic preservatives may have hazardous effects (Krishnakumar and Gordon 1996). The consumer's awareness for "non-chemical" ingredients in health products has also to be faced by the cosmetic and pharmaceutical industries. One of the major emerging technologies is the application of natural additives. The antimicrobial power of plant and herb extracts has been recognized for centuries, and mainly used as natural medicine; however, the trends in using these compounds as food preservatives are increasing nowadays (Ayala-Zavala and González-Aguilar 2011).

It is a common practice in the food industry to remove the desired part of the raw fruit from other nonedible constituents of the plant tissue. The most abundant byproducts of fruit processing are peel, leaves, and seeds. As an example, the desired bean is separated from the fruit skin and other undesirable constituent during the processing of coffee (Vignoli et al. 2011). Likewise, crops are typically valued for their fruits; processing of them involves separating the valuable fruit flesh part from byproducts such as skin and seeds (Ayala-Zavala et al. 2010).

The mass of plant food byproducts obtained as a result of processing crops may approach or even exceed that of the corresponding valuable product affecting the economics of growing crops (Miljkovic and Bignami 2002). In the past, this costly problem has been mitigated to some extent by processing the byproducts further to yield a product that presents less of a disposal problem or that has some marginal economic value (Sun-Waterhouse et al. 2009). In this context, the economics of processing fruits could be improved by developing higher value use for their byproducts. For instance, several patents have been published relating the use of crops as a source of functional compounds (Miljkovic and Bignami 2002; Garrity et al. 2004; Andrews and Andrews 2008). It is known that the byproducts of several fruits contain high levels of organic compounds, the majority of which do not appear to participate directly in growth and development of plant (Croteau et al. 2000). These substances, in many cases, serve as plant defense mechanism to avoid predation by microorganisms, insects, and herbivores. Based on their known mechanism of action, efforts had been directed to evaluate the antimicrobial effect of this kind of compounds, applying them under specific conditions, microorganisms and concentrations, with the goal to find a friendly alternative method to control fungal diseases (Shrikhande 2000; Gorinstein et al. 2001; Troncoso-Rojas and Tiznado-Hernández 2007; Muthuswamy et al. 2008; Tuchila et al. 2008). If this approach is realized, it would be feasible to fulfill the requirements of consumers

for natural and preserved healthy food. In addition, the full utilization of fruits could lead the industry to a lower waste agribusiness, increasing industrial profitability. In this context, the main goal of this chapter is to highlight the agro industrial potential of fruit byproducts as a source of natural antifungal agents, and their possible uses in the food, cosmetic, and pharmaceutical industries.

16.2 Fruit Byproducts: Problematic and Needs

In the horticultural sector, there has been a growth in both acreage and agricultural production to fulfill the requirements of global food demand (Schieber et al. 2001). This intensity of production generates large amount of plant products, estimated to be around 800,000 tons/year of fresh fruits and vegetables, without considering the losses and wastage during processing. The full utilization of horticultural produce is a requirement and a demand that needs to be met by countries wishing to implement low-waste technology in their agribusiness (Kroyer 1995).

As an example, fresh fruits are usually washed, peeled, and sliced to obtain fresh-cut products that retained a high visual and nutritional quality (Robles-Sánchez et al. 2007, Ayala-Zavala et al. 2010). These production steps produce several byproducts, usually skins and seeds, which normally have no further usage and are commonly wasted or discarded (Ajila et al. 2007) affecting the environmental and producing economical lost to producers. Ayala-Zavala et al. (2010) found that several kinds of fresh-cut fruits produced variable amounts of byproducts to the extent even exceeding the quantity of end produce (Table 16.1). In the past, this costly problem has been mitigated to some extent by processing the byproducts further to yield a product that presents less of a disposal problem or that has some marginal economic value (Sun-Waterhouse et al. 2009). In this context, the use of the entire plant tissue, and therefore the developing of higher value use for its byproducts could have economic benefits to producers.

Citrus is one of the most abundant crops in the world, with a worldwide production at around 88×10^6 tons. Among this crop, oranges, lemons, grapefruits, and mandarins are processed in the food industry for juice, but also for jam in the canning industry (Izquierdo and Sendra 2003). As half of the citrus mass is composed of wastes, these processing industries generate over 15×10^6 tons of

Table 16.1 Percentage of recovery of fresh-cut fruits and byproducts

Fresh-cut produce	Final product (%)	Seed (%)	Peel (%)	Other (%)	Total byproduct (%)
Mandarin	83.95		16.05		16.05
Apple	89.09	10.91			10.91
Papaya	52.96	6.51	8.47	32.06	47.04
Pineapple	48.04		13.48	38.48	51.96
Mangoes	57.56	13.50	11.00	17.94	42.44

Ayala-Zavala et al. (2010)

byproducts that some chemical industries used to extract flavonoids and essential oils (EOs) (Marín et al. 2007).

Grapes (*Vitis vinifera* L.) belong to the world's largest fruit crops with a global production of around 69×10^6 tons in 2006 (FAOSTAT 2007). Since about 80 % of the total amount is used in winemaking, some 10 million tons of grapes arise within a few weeks of the harvest campaign. Seeds constitute a considerable proportion of the grape, ranging from 38 to 52 % on a dry matter basis. The seed oil is rich in unsaturated fatty acids (particularly linoleic acid) and phenolic compounds (Schieber et al. 2002; Maier et al. 2009).

Other successful examples of fruits that can show the profitability of using their byproducts are coffee, macadamia, mango, and papaya (Miljkovic and Bignami 2002). Processing of coffee generally involves separating the desired beans from the byproducts of processing e.g., the so-called "coffee cherry", which consists of the fruit skin and other undesirable constituents. On the other hand, macadamia is a tropical exotic fruit that contains an inner and outer shell, and a nut. Processing generally involves separating the valuable nut (main product) from the shells considered as byproducts. Also, pineapple, taro, papaya, and mango are typically appreciated for their flesh but processing of these crops involves separation and removal of the skin and seed byproducts. For instance, U.S. Patent application U.S. 2002/0187239 A1 have proposed the use of coffee cherry, macadamia, mango, taro, and papaya byproducts as a source of nutritional constituents (Miljkovic and Bignami 2002). However, little practical effort to utilize these byproducts has been reported.

However, to evaluate the feasibility of the use of the entire plant tissue, yield and economic parameters involved in the extraction of bioactive compounds from fruit byproducts must be taken into consideration. Characteristics such as pre-treatment requirements, market opportunities and costs must be assessed. With this in mind, some byproducts with remarkable bioactive yield might be regarded as too expensive or of little promise for the market. For instance, Peschel et al. (2006) informed that the high moisture content of fresh byproducts obtained from strawberry, tomato, and apple processing required a cost-intensive drying process for the extraction of bioactive compounds at a temperature below 60 °C to prevent its deterioration through heat and enzymes.

Additionally, byproducts generated during the processing of fruits that are discarded as a waste becomes a source of pollution. It has now been reported that the byproducts of foods contain high levels of residual phenolics (Gorinstein et al. 2011), being necessary to treat these wastes as a specialized residue. High levels of residual phenolics may have adverse environmental impacts mainly because of its properties of seed germination inhibition (Negro et al. 2003). Therefore, industry has increasing cost for its waste treatment. In view of these issues, the use of the entire plant tissue could also have a beneficial impact on the environment.

In view of the mentioned above, several benefits arise from the integral exploitation of the plant tissue. On the one hand, producers would have economic benefits and consumers a greater variety of products. On the other hand, the negative impact that the large amount of wastes have on the environment would be

reduced (Schieber et al. 2001). This situation can be extrapolated to different processing areas, including the food industry, and also the cosmetic and pharmaceutical industries. These three sectors are drawn together and promoted products named functional foods, food supplements, nutraceuticals, or cosmeceuticals (Peschel et al. 2006). However, the integral exploitation of plant produce has not yet been achieved.

16.3 Antifungal Agents in Fruit Processing Byproducts

The presence and growth of fungi in food may cause spoilage and result in a reduction in quality and quantity. So far, many molds, such as *Fusarium* spp., *Aspergillus* spp., *Penicillium* spp. and *Rhizopus* spp., have been reported as the causal agents of foodborne diseases and/or food spoilage (Betts et al. 1999).

It is thought that some chemicals generally used as food preservatives may have a residual toxicity and cause carcinogenic and teratogenic diseases. For these reasons, the demand for natural and socially more acceptable preservatives has been intensified (Skandamis et al. 2001). The exploration of naturally occurring antimicrobials for food preservation receives increasing attention due to consumer awareness of natural food products and a growing concern of microbial resistance toward conventional preservatives (Schuenzel and Harrison 2002). The antimicrobial power of plant and herb extracts has been recognized for centuries, and mainly used as natural medicine.

The most abundant byproducts of minimal processing of fresh-cut fruits are peel, leaves, and seeds and those are reported to contain high amounts of EOs and phenolic compounds with antioxidant and antimicrobial properties (Shrikhande 2000; Gorinstein et al. 2001; Muthuswamy et al. 2008; Tuchila et al. 2008). The content of these functional compounds in different tissues of the fruits depend on the evaluated product but, in general, vitamin C is uniformly distributed in fruits, carotenoids occur mainly on the surface of the tissues such external pericarp and peel, phenolic compounds are located preferentially in peel and seeds and in a lesser extent in the flesh (Kalt 2005) and EOs are concentrated in peel or skin. However, the amount and concentration of individual bioactive compounds is a function of the type of cultivar, the maturity stage of the fruit, the storage conditions and the preharvest handling, and among others.

Recently, there has been considerable interest expressed in EOs from plants with antimicrobial activities for controlling pathogens and toxin-producing microorganisms in foods (Soliman and Badeaa 2002; Tepe et al. 2005). It was reported that 60 % of EOs derivatives examined to date were inhibitory to fungi while 30 % inhibited bacteria (Chaurasia and Vyas 1977). EOs are volatile, natural, and complex compounds characterized by a strong odor and are formed by aromatic plants as secondary metabolites (Bakkali et al. 2008). Numerous studies have documented the antifungal properties of plant EOs (Bouchra et al. 2003; Daferera et al. 2003; Sokmen et al. 2004). These properties are caused by many

active phytochemicals and several volatile compounds, including flavonoids, terpenoids, carotenoids, coumarins and curcumines (Tepe et al. 2005) as well as organic sulfur compounds, aldehydes, alcohols, and among others (Berger 2007). Considering the complex mixture of EOs constituents is difficult to attribute the antimicrobial mode of action to one specific mechanism, being reported several targets in the microbial cell. Some authors mentioned that these compounds may cause damage to the cell wall, the cytoplasmic membrane and the membrane of proteins causing consequently leakage of cell content. Additionally, coagulation of cytoplasm, depletion of proton motive active sites, inactivation of essential enzymes, and disturbance of genetic material functionality properties have been reported (Burt 2004; Ayala-Zavala et al. 2008; Gutierrez et al. 2008). The biological activity of EOs may be of great importance in several fields, from food chemistry to pharmacology and pharmaceutics (Cristani et al. 2007). The main advantage of EOs is that they can be used in any foods and are considered generally recognized as safe (GRAS) (Kabara 1991), as long as their maximum effects are attained with the minimum change in the organoleptic properties of the food.

On the other hand, phenolic compounds are an important class of secondary metabolites with a large range of structures and functions, but generally possessing an aromatic ring bearing one or more hydroxyl substituents (Croteau et al. 2000). Evidence suggests that simple phenolic compounds or phenolic acids, such as tannins, lignans, flavonoids, quinones, coumarines and stilbenes, serve as defenses against herbivores and pathogens and play an important role in disease resistance (Osbourn 1999) and in fruits' protection against spoilage agents, penetrating the cell membrane of microorganisms, causing lysis (Brul and Coote 1999; Ejechi and Akpomedaye 2005). Furthermore, it has also been suggested that the site(s) and number of hydroxyl groups on the phenol group are thought to be related to their antioxidant and antimicrobial capacity and relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased microbial toxicity (Cowan 1999). Plant phenolic compounds that had been reported with antifungal activity are monophenols, di-, and tri-hydroxy phenols, phenolic acids, pterocarpans, isoflavans, isoflavones, isoflavanones, glucosides of isoflavonoids, furanocoumarins, anthocyanidins, tannins, and stilbenes (Vidhyasekaran 1997). Some studies about the antimicrobial activity of phenolic extract from byproducts of fruits have been achieved as it is explained in the next sections. In this context, the fruit byproducts are promising new sources of antimicrobial compounds, offering new commercial opportunities to food and other industries.

The seed and peel of three varieties of avocado (Shepard, Hass and Fuerte) showed activity against yeast (Raymond Chia and Dykes 2010). The seed ethanolic extracts of avocado Hass with a minimum inhibitory concentration value of 104.2 μg/mL was the most effective against *Zygosaccharomyces bailii* but no inhibition was observed against *Penicillium* spp. and *Aspergillus flavus*. Taveira et al. (2010) studied the antimicrobial potential of 10 different tomato seed extracts from "Bull's heart" and "Cherry" varieties, a major byproduct of the tomato processing industry. They analyzed the seed extracts against *Candida albicans*,

Aspergillus fumigatus, and *Trichophyton rubrum*. "Bull's heart" extracts were the most active, *C. albicans* was the most susceptible species (MIC: 5–10 mg/mL).

Pomegranate (*Punica granatum* L.) is a deciduous shrub native to Iran (Sarkhoush et al. 2007). Because of its high antimicrobial activity against many pathogens, pomegranate has been widely used for the treatment of different types of human disease. During the industrial processing of this fruit, large volumes of industrial wastes are produced, which have high antioxidant and antifungal properties (Orzua et al. 2009). Tehranifar et al. (2011) studied the effect of aqueous and methanolic extracts of three different parts of pomegranate (peel, seed, and leaf) with four concentrations (0, 500, 1,000, and 1,500 ppm) on three postharvest fungi (*P. italicum*, *R. stolonifer,* and *B. cinerea*). Based on the results, the methanolic extract showed the highest inhibitory effects on the mycelia growth (IMG) and spore germination (ISG). On the other hand, peel and seed extracts had more inhibitory effect (IMG and ISG) than leaf extract. The phenolic content of peel extract was also measured 2.8-fold higher than pomegranate leaf extract. The authors concluded that the high percentage of phenolic content in the peel and seed of pomegranate could cause the high antifungal activity of their extracts.

Mandalari et al. (2007) evaluated a flavonoid-rich extract from the peel of Bergamot citrus fruit, an important byproduct in the processing industry, against the yeast *Saccharomyces cerevisiae*. They found that the antimicrobial potency of the bergamot extracts increased after enzymatic deglycosylation and that the aglycone eriodictyol was the most active against *S. cerevisiae*.

The EOs of lemon (*Citrus lemon* L.), mandarin (*Citrus reticulata* L.), grapefruit (*Citrus paradisi* L.), and orange (*Citrus sinensis* L.) obtained by cold pressing the peel and tested at different concentrations (0.47, 0.27, 0.94, and 0.71 %), showed the capacity to reduce or inhibit the growth of the molds *P. chrysogenum*, *P. verrucosum*, *A. niger,* and *A. flavus* (Viuda-Martos et al. 2008). In the case of *A. niger*, orange EO produced the greatest reduction in mycelium growth followed by lemon EO. With *A. flavus*, total inhibition of growth was obtained with all the EOs at the highest concentration of 0.94 %. In this case, though, the mandarin EO showed the highest inhibitions of mycelial growth. Grapefruit was the most effective EO in reducing the growth of *P. chrysogenum* and *P. verrucosum* while lemon was the next best EO.

Okwu et al. (2007) also studied the antifungal activity of five varieties of citrus species: sweet orange (*Citrus sinensis*), tangerine (*Citrus reticulata*), lemon (*Citrus limonum*), lime (*Citrus aurantifolia*), and grape (*Citrus grandis*) against *Fusarium oxysporum*. The growth of this fungi was inhibited *in vitro* by the extracts of the citrus species. The extracts of the peels of *C. sinensis*, *C. aurentifolia,* and *C. reticulata* showed 83.55, 71.10, and 68.14 % inhibition activity, respectively. The authors found that the fungitoxicity of the extracts from the peels of *C. sinensis* was the same as that of benomyl, a synthetic fungicide.

Several authors have attributed the antifungal capacity of citrus EOs to the presence of components such as D-limonene, linalool, or citral (Rodov et al. 1995; Rasooli et al. 2002; Alma et al. 2004; Bezić et al. 2005; Sonboli et al. 2006; Tepe et al. 2006), which are present in differing concentrations in citric EOs (Veriotti

and Sacks 2001; Vekiari et al. 2002). Other author attributed this function to the phenolic compounds: the amphipathicity of these compounds can explain their interactions with biomembrane and thus the antimicrobial activity (Veldhuizen et al. 2006).

Flavanones are a particular class of flavonoids commonly found in citrus leaves as glycosides. The most common citrus flavanone glycosides are hesperidin or 3',5,7-trihydroxy-4'-methoxyflavanone-7-α-L-rhamnosyl(1 → 6)-β-D-glucoside, which is found in oranges, lemons and other citrus, naringin or 3',5,7-trihydroxy-4'-methoxyflavanone-7-α-L-rhamnosyl(1 → 6)-β-D-glucoside in grapefruits and sour oranges and neohesperidin or 3',5,7-trihydroxy-4'-methoxyflavanone-7-α-L-rhamnosyl(1 → 2)-β-D-glucoside in sour oranges. This variety of flavonoids has been obtained as a byproduct of the citrus industries (Ellenrieder 2004). Salas et al. (2011) studied the antifungal activity of 10 isolated flavonoids from citrus species on four fungi often found as food contaminants: *Aspergillus parasiticus*, *A. flavus*, *Fusarium semitectum,* and *Penicillium expansum*. All flavonoids, at 0.25 mM, showed the capacity to alter the growth of fungi tested but at this concentration total inhibition was not achieved. The intensity of the antifungal activity depended on the type of fungus and compound used. The hesperetin glucoside laurate strongly inhibited the mycelial growth of *P. expansum*, while prunin decanoate was the most inhibiting flavonoid for *A. flavus*, *A. parasiticus*, and *F. semitectum*.

16.4 Conventional and Future Uses of Antifungal Compounds from Fruit Byproducts

Within the literature, the number of studied residual sources has been augmented considerably, which is caused by a value adding recycling interest of the agro and food industry, but also increasing information on the specific location of active compounds and their modification during processing (Peschel et al. 2006). However, only a few byproduct derived bioactive compounds have been developed successfully from the vast quantities of plant residues produced by the food processing industry, primarily grape seed, olive waste extracts (Alonso et al. 2002; Amro et al. 2002), and citrus residues (Marín et al. 2007). Little practical effort to utilize the byproducts of fruits with a high annual production and a high content of bioactive compounds (such as apple, tomato, strawberry, and pear) has been reported. This might be caused by three limiting factors often overlooked in scientific studies: the effectiveness of recovery and extraction, the marketability of resulting extracts, and the practical suitability for the food, cosmetic or pharmaceutical products (Peschel et al. 2006). In the next sections, a review about the utilization of fruit byproducts as antimicrobials agents in the food industry is presented. Additionally, possible utilization of fruit wastes for the cosmetic and pharmaceutical industries are analyzed.

The usage of bioactive extracts as applied to food preservation is an alternative to chemical preservatives and helps to achieve consumer demand for fresh, nutritious, and safe items that are free of synthetic additives. Presently, there are studies that provide information about the effect of bioactive compounds that are extracted from plant extracts and applied to food (Lanciotti et al. 2004; Tripathi and Dubey 2004; Guillen et al. 2007; Muthuswamy and Vasantha Rupasinghe 2007; Martín-Diana et al. 2008; Muthuswamy et al. 2008; Raybaudi-Massilia et al. 2009). However, the effect of antifungal obtained from fruit byproducts as food antifungal preservatives has not been extensively reported. As mentioned elsewhere, the citrus industry produced large amounts of byproducts. Oils obtained from skin have been used for different applications. Studies of the application of lemon extract on dairy products have also been performed (Conte et al. 2007). In this case, different antimicrobial packaging systems including lemon extracts have been used to preserve Mozzarella cheese. Results showed an increase in the shelf life of all active packaged Mozzarella cheeses, confirming that lemon extract may exert an inhibitory effect on the microorganisms responsible for spoilage phenomena without affecting the functional microbiota of the product.

Ponce et al. (2003) evaluated the inhibitory effects of essential lemon oil on the native microbial populations of Swiss chard. The essential oil of lemon yielded a minimum inhibitory concentration of about 0.05/100 mL and the minimum antimicrobial concentration was not reached at the highest concentration tested, indicating that high concentrations of this oil would be needed to achieve effects. The antimicrobial potentials of pomegranate peel extract were investigated in chicken products (Li et al. 2006; Kanatt et al. 2010). Pomegranate peel extract showed good antimicrobial activity against *Bacillus cereus*. In general, addition of pomegranate peel extract to popular chicken and meat products enhanced its shelf life by 2–3 weeks, during chilling temperature storage. Although some bioactive extracts from fruit byproducts have been proven to be effective antimicrobials, it is important to note that its addition to fruits may cause changes in sensorial attributes. Therefore, undesirable sensorial effects can be a limiting factor and careful selection of type and concentration according to the type of food must be considered (Burt 2004).

In another context, the burgeoning consumer demand of the past decade for natural food additives has prompted the revision of regulatory actions governing the use of natural plant extracts as food additives (Marriott 2010). As an example, the regulation EC/1334/2008, introduced in January 2011 to the European legislation, contains new definitions for natural extracts and processes that can be used in their preparation.

Due to the preserving activity of EOs, these substances could also be applied for the preservation of cosmetic products. Since some EOs show synergistic effects in combination with commercially used preservatives, the application of EOs makes a diminution of these synthetic substances possible as the study mentioned below revealed (Kunicka-Styczyńska et al. 2009; Patrone et al. 2010). The application of lemon EOs in body milks was investigated observing the inhibition of microbial growth. The most abundant substance in lemon oil was limonene (79.8 %). The

growth of the involved microorganisms *S. aureus*, *P. aeruginosa*, *Aspergillus niger,* and *Candida* species was sufficiently inhibited using the lemon EOs in combination with 0.2 % of a synthetic preservative. Since synergy was noticed when the EOs were combined with the synthetic agent, the applied quantity of the synthetical component could be cut down about 8.5 times (Kunicka-Styczyńska et al. 2009). It can also be of interest for the cosmetic industry, the application of natural bioactive compounds is a substitute for synthetic preservatives or as active ingredients, for example, a skin-protecting additive in dermatology.

In the past few decades, a worldwide increase in the incidence of fungal infections has been observed as well as a rise in the resistance of some species of fungus to different fungicidals used in medicinal practice (Abad et al. 2007). Candidiasis and cryptococcosis of continuously expanding global incidence are a result of increased immunosuppressive disorders, including AIDS and certain chemo- or radiotherapies (Sobel et al. 2004). The majority of clinically used antifungals have various drawbacks in terms of toxicity, efficacy and cost, and their frequent use has led to the emergence of resistant strains.

As we have seen previously, natural compounds, including phenolics and EOs obtained from fruits, are a potential source of antimycotic agents either in their nascent form or as template structures for more effective derivatives and are considered to be innately safe for humans.

16.5 Conclusion

The analyzed information showed that bioactive compounds from fruit byproducts could be used as natural antifungal additives to offer protection to food. If this approach is realized, it would be possible to fulfill the requirements of the consumers in natural and preserved healthy and convenient food, and the full utilization of the fruits could lead the industry to a lower waste agribusiness, increasing industrial profitability through environmentally friendly operating processes. Additionally, its antioxidant and antifungal properties are of particular interest in other sectors like cosmetic and pharmaceutical industries.

We consider that future research and development efforts on the treated topic must be undertaken. From the point of view of legislation, the international regulations on the use of plant extracts as food additives must be improved. On the other hand, the industry has to performed toxicological analysis of bioactive extracts, studies on the metabolism of bioactive compounds, their bioavailability and bioaccessibility, and the sensorial and nutritional aspects of the food and other kind of products added with bioactive compounds from fruit residues. In addition, the analysis of the economic feasibility of the process of extraction of bioactive compounds and the evaluation of the effect of the extraction procedure (solvents, temperature, and raw material) on the composition and activity of the obtained extracts, identifying the optimal application procedure and required doses to achieve antimicrobial fortification without affecting sensorial acceptability must be

carry out. Additionally, odor–flavor masking technologies could be contemplated and investigated if the bioactive compounds affect the sensorial acceptability of the product. Some of them include the incorporation of extracts in edible coatings, encapsulation technologies, and controlled release systems. Marketing of natural bioactive extracts must also be contemplated. Likewise, practical and legal questions required interdisciplinary cooperation of academia and industry in this field. The bioactive compounds of fruit byproducts represent a new class of functional foods that has not been completely exploited and that could also contribute to different health benefits to consumers.

References

Abad MJ, Ansuategui M, Bermejo P (2007) Active antifungal substances from natural sources. Arkivoc 7:116–145

Ajila C, Bhat S, Prasada Rao U (2007) Valuable components of raw and ripe peels from two Indian mango varieties. Food Chem 102(4):1006–1011

Alma MH, Nitz S, Kollmannsberger H, Digrak M, Efe FT, Yilmaz N (2004) Chemical composition and antimicrobial activity of the essential oils from the gum of Turkish pistachio (*Pistacia vera* L.). J Agric Food Chem 52(12):3911–3914

Alonso ÁM, Guillén DA, Barroso CG, Puertas B, García A (2002) Determination of antioxidant activity of wine byproducts and its correlation with polyphenolic content. J Agric Food Chem 50(21):5832–5836

Amro B, Aburjai T, Al-Khalil S (2002) Antioxidative and radical scavenging effects of olive cake extract. Fitoterapia 73(6):456–461

Andrews DA, Andrews K (2008) Nutraceutical moringa composition. Google patents Appl. no.: 12/338,789, Pub. no.: US 2009/0098230 A1, Pub, 18 Dec 2008

Ayala-Zavala JF, González-Aguilar GA (2011) Use of additives to preserve the quality of fresh-cut fruits and vegetables. In: Martín-Belloso O, Soliva-Fortuny R (eds) Advances in fresh-cut fruits and vegetables processing. CRC Press, Boca Raton, pp 231–254

Ayala-Zavala J, Del-Toro-Sánchez L, Alvarez-Parrilla E, González-Aguilar G (2008) High relative humidity in-package of fresh-cut fruits and vegetables: advantage or disadvantage considering microbiological problems and antimicrobial delivering systems? J Food Sci 73(4):R41–R47

Ayala-Zavala J, Rosas-Domínguez C, Vega-Vega V, González-Aguilar G (2010) Antioxidant enrichment and antimicrobial protection of fresh-cut fruits using their own byproducts: looking for integral exploitation. J Food Sci 75(8):R175–R181

Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008) Biological effects of essential oils–a review. Food Chem Toxicol 46(2):446–475

Berger RG (2007) Flavours and fragrances: chemistry bioprocessing and sustainability. Springer, Berlin

Betts G, Linton P, Betteridge R (1999) Food spoilage yeasts: effects of pH, NaCl and temperature on growth. Food Control 10(1):27–33

Bezić N, Skočibušić M, Dunkić V (2005) Phytochemical composition and antimicrobial activity of *Satureja montana* L. and *Satureja cuneifolia* Ten. essential oils. Acta Bot Croat 64(2):313–322

Bouchra C, Achouri M, Idrissi Hassani L, Hmamouchi M (2003) Chemical composition and antifungal activity of essential oils of seven Moroccan Labiatae against *Botrytis cinerea* Pers: Fr. J Ethnopharmacol 89(1):165–169

Brul S, Coote P (1999) Preservative agents in foods: mode of action and microbial resistance mechanisms. Int J Food Microbiol 50(1):1–17

Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods—a review. Int J Food Microbiol 94(3):223–253

Chaurasia S, Vyas K (1977) In vitro effect of some volatile oil against *Phytophthora parasitica* var. piperina. J Res Indian Med Yoga Homeopath 1977:24–26

Conte A, Scrocco C, Sinigaglia M, Del Nobile M (2007) Innovative active packaging systems to prolong the shelf life of mozzarella cheese. J Dairy Sci 90(5):2126–2131

Cowan MM (1999) Plant products as antimicrobial agents. Clin Microbiol Rev 12(4):564–582

Cristani M, D'Arrigo M, Mandalari G, Castelli F, Sarpietro MG, Micieli D, Venuti V, Bisignano G, Saija A, Trombetta D (2007) Interaction of four monoterpenes contained in essential oils with model membranes: implications for their antibacterial activity. J Agric Food Chem 55(15):6300–6308

Croteau R, Kutchan TM, Lewis NG (2000) Natural products (secondary metabolites). In: Buchanan B, Gruissem W, Jones R (eds) Biochemistry and molecular biology of plants. American Society of Physiologists, Rockville, MD, USA, pp. 1250–1318

Daferera DJ, Ziogas BN, Polissiou MG (2003) The effectiveness of plant essential oils on the growth of *Botrytis cinerea*, *Fusarium* sp. and *Clavibacter michiganensis* subsp. michiganensis. Crop Prot 22(1):39–44

Ejechi B, Akpomedaye D (2005) Activity of essential oil and phenolic acid extracts of pepperfruit (*Dennetia tripetala G. Barker; Anonaceae*) against some food-borne microorganisms. Afr J Biotechnol 4(3):258–261

Ellenrieder G (2004) Biotransformations of citrus flavanone glycosides. In: Pandey A (ed) Concise encyclopedia of bioresource technology. Food Products Press, New York, pp 189–199

FAOSTAT (2007) FAO Statistical Database. http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor

Garrity AR, Morton GA, Morton JC (2004) Nutraceutical mangosteen composition. Google Patents, Patent No.: US 6730333 B1, Appl. No.: 10/283,600, 4 May 2004

Gorinstein S, Martin-Belloso O, Park YS, Haruenkit R, Lojek A, Cíz M, Caspi A, Libman I, Trakhtenberg S (2001) Comparison of some biochemical characteristics of different citrus fruits. Food Chem 74(3):309–315

Gorinstein S, Poovarodom S, Leontowicz H, Leontowicz M, Namiesnik J, Vearasilp S, Haruenkit R, Ruamsuke P, Katrich E, Tashma Z (2011) Antioxidant properties and bioactive constituents of some rare exotic Thai fruits and comparison with conventional fruits: *in vitro* and *in vivo* studies. Food Res Int 44(7):2222–2232

Guillen F, Zapata P, Martínez-Romero D, Castillo S, Serrano M, Valero D (2007) Improvement of the overall quality of table grapes stored under modified atmosphere packaging in combination with natural antimicrobial compounds. J Food Sci 72(3):S185–S190

Gutierrez J, Barry-Ryan C, Bourke P (2008) The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. Int J Food Microbiol 124(1):91–97

Izquierdo L, Sendra J (2003) Citrus fruits. composition and characterization. In: Caballero LTB, Finglas P (eds) Encyclopedia of food sciences and nutrition, 2nd edn. Academic Press, Oxford, pp 1335–1341

Kabara J (1991) Phenols and chelators. In: Russell NJ, Gould GW (eds) Food preservatives. Blackie, London, pp 200–214

Kalt W (2005) Effects of production and processing factors on major fruit and vegetable antioxidants. J Food Sci 70(1):R11–R19

Kanatt SR, Chander R, Sharma A (2010) Antioxidant and antimicrobial activity of pomegranate peel extract improves the shelf life of chicken products. Int J Food Sci Technol 45(2):216–222

Krishnakumar V, Gordon I (1996) Antioxidants–trends and developments. Int Food Ingredients 12:41–44

Kroyer GT (1995) Impact of food processing on the environment—an overview. LWT-Food Sci Technol 28(6):547–552

Kunicka-Styczyńska A, Sikora M, Kalemba D (2009) Antimicrobial activity of lavender, tea tree and lemon oils in cosmetic preservative systems. J Appl Microbiol 107(6):1903–1911

Lanciotti R, Gianotti A, Patrignani F, Belletti N, Guerzoni M, Gardini F (2004) Use of natural aroma compounds to improve shelf-life and safety of minimally processed fruits. Trends Food Sci Technol 15(3):201–208

Li Y, Guo C, Yang J, Wei J, Xu J, Cheng S (2006) Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Food Chem 96(2):254–260

Maier T, Schieber A, Kammerer DR, Carle R (2009) Residues of grape (*Vitis vinifera* L.) seed oil production as a valuable source of phenolic antioxidants. Food Chem 112(3):551–559

Mandalari G, Bennett R, Bisignano G, Trombetta D, Saija A, Faulds C, Gasson M, Narbad A (2007) Antimicrobial activity of flavonoids extracted from bergamot (*Citrus bergamia Risso*) peel, a byproduct of the essential oil industry. J Appl Microbiol 103(6):2056–2064

Marín FR, Soler-Rivas C, Benavente-García O, Castillo J, Pérez-Alvarez JA (2007) By-products from different citrus processes as a source of customized functional fibres. Food Chem 100(2):736–741

Marriott R (2010) Flavours-Greener chemistry preparation of traditional flavour extracts and molecules. Agro Food Ind Hi Tec 21(2):46

Martín-Diana AB, Rico D, Barry-Ryan C (2008) Green tea extract as a natural antioxidant to extend the shelf-life of fresh-cut lettuce. Innov Food Sci Emerg Technol 9(4):593–603

Miljkovic D, Bignami GS (2002) Nutraceuticals and methods of obtaining nutraceuticals from tropical crops. Google Patents, Appl. No.: 10/067,569, Pub. No.: US 2002/0187239 A1, Pub, 12 Dec 2002

Muthuswamy S, Vasantha Rupasinghe H (2007) Fruit phenolics as natural antimicrobial agents: selective antimicrobial activity of catechin, chlorogenic acid and phloridzin. J Food Agric Environ 5(3–4):81–85

Muthuswamy S, Rupasinghe H, Stratton G (2008) Antimicrobial effect of cinnamon bark extract on *Escherichia coli* O157: H7, *Listeria innocua* and fresh-cut apple slices. J Food Saf 28(4):534–549

Negro C, Tommasi L, Miceli A (2003) Phenolic compounds and antioxidant activity from red grape marc extracts. Bioresour Technol 87(1):41–44

Okwu DE, Awurum AN, Okoronkwo JJ (2007) Phytochemical composition and in vitro antifungal activity screening of extracts from citrus plants against Fusarium oxysporum of okra plant (Hibiscus esculentus). Afr Crop Sci Conf Proc 8:1755–1758

Orzua MC, Mussatto SI, Contreras-Esquivel JC, Rodriguez R, De la Garza H, Teixeira JA, Aguilar CN (2009) Exploitation of agro industrial wastes as immobilization carrier for solid-state fermentation. Ind Crop Prod 30(1):24–27

Osbourn AE (1999) Antimicrobial phytoprotectants and fungal pathogens: a commentary. Fungal Genet Biol 26(3):163–168

Patrone V, Campana R, Vittoria E, Baffone W (2010) In vitro synergistic activities of essential oils and surfactants in combination with cosmetic preservatives against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Curr Microbiol 60(4):237–241

Peschel W, Sánchez-Rabaneda F, Diekmann W, Plescher A, Gartzía I, Jiménez D, Lamuela-Raventos R, Buxaderas S, Codina C (2006) An industrial approach in the search of natural antioxidants from vegetable and fruit wastes. Food Chem 97(1):137–150

Ponce A, Fritz R, Del Valle C, Roura S (2003) Antimicrobial activity of essential oils on the native microflora of organic Swiss chard. LWT-Food Sci Technol 36(7):679–684

Rasooli I, Moosavi M, Rezaee M, Jaimand K (2002) Susceptibility of microorganisms to *Myrtus communis* L. essential oil and its chemical composition. J Agric Sci Technol 4:127–133

Raybaudi-Massilia RM, Mosqueda-Melgar J, Soliva-Fortuny R, Martín-Belloso O (2009) Control of pathogenic and spoilage microorganisms in fresh-cut fruits and fruit juices by traditional and alternative natural antimicrobials. Compr Rev Food Sci Food Saf 8(3):157–180

Raymond Chia TW, Dykes GA (2010) Antimicrobial activity of crude epicarp and seed extracts from mature avocado fruit (*Persea americana*) of three cultivars. Pharm Biol (Formerly Int J Pharmacog) 48(7):753–756

Robles-Sánchez M, Gorinstein S, Martín-Belloso O, Astiazarán-García H, González-Aguilar G, Cruz-Valenzuela R (2007) Frutos tropicales mínimamente procesados: Potencial antioxidante y su impacto en la salud. INCI 32(4):227–232

Rodov V, Ben-Yehoshua S, Fang DQ, Kim JJ, Ashkenazi R (1995) Preformed antifungal compounds of lemon fruit: citral and its relation to disease resistance. J Agric Food Chem 43(4):1057–1061

Salas MP, Céliz G, Geronazzo H, Daz M, Resnik SL (2011) Antifungal activity of natural and enzymatically-modified flavonoids isolated from citrus species. Food Chem 124(4):1411–1415

Sarkhoush A, Zamani Z, Fatahi R, Ghorbani H, Hadia J (2007) A review on medicinal characteristics of pomegranate (*Punica granatum* L.). J Med Plants 6(22):13–24

Schieber A, Stintzing F, Carle R (2001) By-products of plant food processing as a source of functional compounds–recent developments. Trends Food Sci Technol 12(11):401–413

Schieber A, Müller D, Röhrig G, Carle R (2002) Effects of grape cultivar and processing on the quality of cold-pressed grape seed oils. Mitt Klosterneuburg 52:29–33

Schuenzel KM, Harrison MA (2002) Microbial antagonists of foodborne pathogens on fresh, minimally processed vegetables. J Food Prot 65(12):1909–1915

Shrikhande AJ (2000) Wine by-products with health benefits. Food Res Int 33(6):469–474

Skandamis P, Koutsoumanis K, Fasseas K, Nychas GJE (2001) Inhibition of oregano essential oil and EDTA on *Escherichia coli* O157: H7. Ital J Food Sci 13(1):65–75

Sobel JD, Wiesenfeld HC, Martens M, Danna P, Hooton TM, Rompalo A, Sperling M, Livengood C III, Horowitz B, Von Thron J (2004) Maintenance fluconazole therapy for recurrent vulvovaginal candidiasis. New Engl J Med 351(9):876–883

Sokmen A, Gulluce M, Askin Akpulat H, Daferera D, Tepe B, Polissiou M, Sokmen M, Sahin F (2004) The in vitro antimicrobial and antioxidant activities of the essential oils and methanol extracts of endemic *Thymus spathulifolius*. Food Control 15(8):627–634

Soliman K, Badeaa R (2002) Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. Food Chem Toxicol 40(11):1669–1675

Sonboli A, Babakhani B, Mehrabian A (2006) Antimicrobial activity of six constituents of essential oil from Salvia. Z Naturforsch C 61(3–4):160–164

Sun-Waterhouse D, Wen I, Wibisono R, Melton LD, Wadhwa S (2009) Evaluation of the extraction efficiency for polyphenol extracts from by-products of green kiwifruit juicing. Int J Food Sci Technol 44(12):2644–2652

Taveira M, Silva LR, Vale-Silva LA, Pinto E, Valentão P, Ferreres F, Guedes de Pinho P, Andrade PB (2010) *Lycopersicon esculentum* seeds: an industrial byproduct as an antimicrobial agent. J Agric Food Chem 58(17):9529–9536

Tehranifar A, Selahvarzi Y, Kharrazi M, Bakhsh VJ (2011) High potential of agro-industrial by-products of pomegranate (*Punica granatum* L.) as the powerful antifungal and antioxidant substances. Ind Crop Prod 34(3):1523–1527

Tepe B, Daferera D, Sokmen A, Sokmen M, Polissiou M (2005) Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae). Food Chem 90(3):333–340

Tepe B, Akpulat HA, Sokmen M, Daferera D, Yumrutas O, Aydin E, Polissiou M, Sokmen A (2006) Screening of the antioxidative and antimicrobial properties of the essential oils of *Pimpinella anisetum* and *Pimpinella flabellifolia* from Turkey. Food Chem 97(4):719–724

Tripathi P, Dubey N (2004) Exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruit and vegetables. Postharv Biol Technol 32(3):235–245

Troncoso-Rojas R, Tiznado-Hernández M (2007) Natural compounds to control fungal postharvest diseases. In: Troncoso-Rojas R, Tiznado-Hernández M, González-León A (eds) Recent advances in alternative postharvest technologies to control fungal diseases in fruits and vegetables. Research Sign Post, Trivandrum, pp 127–156

Tuchila C, Jianu I, Rujescu CI, Butur M, Ahmadi-Khoie M, Negrea I (2008) Evaluation of the antimicrobial activity of some plant extracts used as food additives. J Food Agric Environ 6(3–4):68–70

Vekiari SA, Protopapadakis EE, Papadopoulou P, Papanicolaou D, Panou C, Vamvakias M (2002) Composition and seasonal variation of the essential oil from leaves and peel of a Cretan lemon variety. J Agric Food Chem 50(1):147–153

Veldhuizen EJA, Tjeerdsma-van Bokhoven JLM, Zweijtzer C, Burt SA, Haagsman HP (2006) Structural requirements for the antimicrobial activity of carvacrol. J Agric Food Chem 54(5):1874–1879

Veriotti T, Sacks R (2001) High-speed GC and GC/time-of-flight MS of lemon and lime oil samples. Anal Chem 73(18):4395–4402

Vidhyasekaran P (1997) Phytoalexins. In: Vidhyasekaran P (ed) Fungal pathogenesis in plants and crops: molecular biology and host defense mechanisms. Marcel Dekker, New York, pp 380–455

Vignoli J, Bassoli D, Benassi M (2011) Antioxidant activity, polyphenols, caffeine and melanoidins in soluble coffee: The influence of processing conditions and raw material. Food Chem 124(3):863–868

Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, Pérez-Álvarez J (2008) Antifungal activity of lemon (*Citrus lemon* L.), mandarin (*Citrus reticulata* L.), grapefruit (*Citrus paradisi* L.) and orange (*Citrus sinensis* L.) essential oils. Food Control 19(12):1130–1138

White J, McFadden J (2008) Contact allergens in food ingredients and additives: atopy and the hapten-atopy hypothesis. Contact Dermatitis 58(4):245–246

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