Chapter 10
Volatile Organic Compounds and Their Capacity for Controlling Fungal Infection in Humans

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Abstract It is estimated that over 30% of the world's population has ever had a fungal infection. The most common fungal diseases are nail and skin infections, which are mainly caused by fungi of the genus *Trichophyton* spp., *Epidermophyton* spp., or *Microsporum* spp. Onychomycosis is a fungal infection that affects mainly nails, but can also cause foot and leg ulcerations, leading to extreme situations, such as limb amputation. Among dermatophytes, *T. rubrum* is mainly responsible for skin infections. Antifungal compounds, such as azoles, allylamines, and amorolfine (administered orally or topically), are usually used in the treatment of this disease. The oral mucosa and the genital tract are other targets of attack by fungal pathogens. In these cases, yeasts of the genus *Candida* spp. play a crucial role. It is estimated that about 75% of women have suffered *Candida* spp. vulvovaginitis at least once in their lives. Other opportunistic pathogenic fungi that frequently affect human health are some species belonging to the genus *Cryptococcus* spp. (cutaneous mycoses and opportunistic mycoses) and *Aspergillus* spp. (allergic reactions, keratitis and opportunistic onychomycosis). The usual problem for the treatment of these infections is

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the fungal resistance to azoles, polyenes, echinocandins, among other existing drugs. In this context, there is an urgent need to find out new alternatives for the treatment of fungal infections. Natural products, such as volatile organic compounds, essential oils, or their components, have shown promising antifungal activities. In this chapter, we will discuss the latest findings about the antifungal activity of volatile organic compounds, essential oils, and their individual components against fungi of clinical importance. Moreover, the structure related to the antifungal activity of these natural compounds, their mechanisms of action, and their synergistic properties will also be explored.

Keywords Dermatophytes · *Candida* · Opportunistic fungi · Volatile organic compounds · Terpenoids · Antifungal properties

Abbreviations

AIDS	Acquired immunodeficiency syndrome
EO(s)	Essential oil(s)
FICI	Fractional Inhibitory Concentration Index
ISO	International Organization for Standardization
ISOE	International Standard Organization on Essential Oils (ISOE)
LUMO	Lowest Unoccupied Molecular Orbital
MIC	Minimum inhibitory concentration
MLR	Multiple linear regression analyses
QSAR	Quantitative structure–activity relationship
RMSPE	Root mean square prediction error
SAPs	Secreted aspartic proteases
SAR	structure–activity relationship

1 Introduction

Fungal infections affect more than 30% of the world population (Hameed and Fatima 2019). The incidence of dermatophytosis, like onychomycosis, or superficial mycoses, such as tinea pedis or tinea cruris, has increased in recent years, and in many patients they manifest as chronic or recurrent diseases (Arora et al. 2020). Dermatophytosis and other fungal infections frequently occur in immunocompromised patients, such as those with acquired immunodeficiency syndrome (AIDS), immunologically impaired transplant patients, or patients that are being treated for more critical diseases like cancer (Cassella et al. 2002). Although dermatophytosis mainly affects adult men (Alessandrini et al. 2020), it shows a strong incidence with high morbidity and mortality in pediatric and geriatric populations of both genders (Khan et al. 2014; Gupta et al. 2020). Dermatophytosis is transmitted directly

between people (anthropophilic), by interaction with pets (zoophilic), soil material (geophilic), or indirectly through sharing clothes, brushes, and towels (Arora et al. 2020). Even when they are not life-threatening, superficial fungal infections have several psycho-social effects on patients, affecting their quality of life (Alessandrini et al. 2020). This reality has led to abandon the idea that superficial mycoses are a problem related to beauty; instead, they should be considered as an important public health problem (Bouyahya et al. 2018; Arora et al. 2020)

Onychomycosis is the most important dermatophytosis due to the large number of patients that suffer from this disease (Bouyahya et al. 2018; Alessandrini et al. 2020). Onychomycosis is caused by keratinophilic fungi producers of proteases that break keratin allowing infection of the stratum corneum of the skin, the hair shaft, and the nail (both toenails and fingernails) (Alessandrini et al. 2020). This disease requires long-term treatments, like other fungal diseases. Derivatives of morpholine, azoles, and allylamines are the most frequently used compounds for their control; however, they present dangerous hazardous side effects in many patients, such as high hepatotoxicity, alopecia, among other health problems (Alessandrini et al. 2020; Hameed and Fatima 2019; Marx-Stoelting et al. 2020). For these reasons, topical treatments are preferred since they are associated with low risk of adverse side effects (Alessandrini et al. 2020). The non-responsible use of antifungal drugs causes the raising of resistant strains, which leads to an increase in the treatment doses, hence producing stronger side effects (Baptista et al. 2015; Padovan et al. 2019; Bouyahya et al. 2020). Among the anthropophilic dermatophytes, we can find *Trichophyton rubrum* and *Trichophyton interdigitale* (Alessandrini et al. 2020), *Microsporum* spp., and *Epidermophyton* spp. (Arora et al. 2020). Among the non-dermatophyte fungi that cause mycosis in humans or animals we can find *Scopulariopsis brevicaulis* and species belonging to the genera *Fusarium* spp., *Aspergillus* spp., *Acremonium* spp., *Alternaria* spp., and *Scytalidium* spp. (Alessandrini et al. 2020).

In recent years, the number of scientific reports that assessed natural substances for the development of new antifungals has significantly increased, mainly because many of them produced no side effects (Bouyahya et al. 2020). Among natural products, essential oils (EOs) have shown promising results for the treatment of various diseases such as cancer (Najar et al. 2020), hypertension (Oboh et al. 2017), and diabetes (Lari et al. 2020). The EOs have also proved effective as antibacterial (Zygadlo et al. 2017), antifungal (Pizzolitto et al. 2020; Achimón et al. 2021), antiparasitic (Azadbakht et al. 2020), anti-inflammatory (Yeh and Lin 2020), and antioxidants (Raspo et al. 2020). These medicinal and pharmacological properties are related to the chemical composition of EOs and to the lipophilic nature of the EO molecules (Baptista et al. 2015; Zygadlo et al. 2017). The combination of EOs or their bioactive components with synthetic antifungal drugs enable the reduction of drug concentration, thus decreasing its side effects (Lopes et al. 2017; Ali-Shtayeh et al. 2019). This combination of conventional antifungals and EOs or their components has shown many successful results in treating microorganisms that have developed multi-drug resistance (Lopes et al. 2017). This is very important in diseases such as dermatophytosis where the possibility of recurrence is very frequent, which is why treatments are prolonged over time. Decreasing doses is a main point in patient care to avoid chronic toxicity due to the action of the drugs.

2 Essential Oils and Their Components as Anti-dermatophytic Compounds

Antifungal drugs used for the control of dermatophytes comprise econazole, ketoconazole, fluconazole, itraconazole, miconazole, thioconazole, and clotrimazole. The most frequently used allylamins are terbinafine and naftifine, while the derivatives of morpholine amorolfine and butenafine are the third group of most widely used drugs (Gupta et al. 2020; Hameed and Fatima 2019). Combinations of these anti-dermatophytic drugs have also been performed to synergize their effects and break resistance in microorganisms, hence decreasing their dose (Martinez-Rossi et al. 2018; Padovan et al. 2019; Da Costa et al. 2020). Essential oils are plant-derived-metabolites, often lipophilic and liquid at room temperature, which are stored in specialized glands. The extraction of EOs can be carried out by using different techniques (Zygadlo 2011). The International Standard Organization on Essential Oils (ISOE) states that the extractive technique to obtain EOs from their vegetable sources must be by hydrodistillation of the aromatic plant (Hameed and Fatima 2019). Essential oils are a mixture of monoterpenes, phenylpropanoids, sesquiterpenes, and diterpenes. The functional groups of these compounds are usually diverse; we can find phenols, alcohols, ketones, ethers, aldehydes, and esters (Zygadlo 2011). This enormous chemical diversity of EOs can provide a broad spectrum of biological activities (Chan et al. 2016; Lari et al. 2020; Lira et al. 2020; Najar et al. 2020). Although most bibliographic information indicates that the main action site of EOs or their components is the plasma membrane, the great chemical diversity of these mixtures leads us to think of a multi-target spectrum that involves many mechanisms of action (Buriani et al. 2020). The type of climate, the composition and structure of the soil, the age of the aromatic plants, the organ that produces the EOs, the vegetative state of the plant are some of the variables that also cause great diversity in the composition of EOs (Zygadlo 2011). This list still increases with the development of new cultivars of aromatic plants. For these reasons, to avoid inconveniences in the international trade when marketing and selecting an EO, they must be standardized and comply with quality and composition standards determined by the International Organization for Standardization (ISO) (Zygadlo 2011). This standardization does not exist for other natural compounds and must be performed under the governmental laws of each country, which regulate compounds with pharmacological properties. Hence, the EO that is marketed under ISO standards has its composition guaranteed, which would allow an almost immediate use in the development of antifungal formulations. Many EOs have been evaluated for their antifungal properties (Donato et al. 2020) and their bioactivity against resistant strains (Gallucci et al. 2014), showing strong synergizing properties when combined with synthetic antifungals (Amber et al. 2010; Da Costa et al. 2020; Nogueira Sobrinho et al. 2020). In any case, few studies have been carried out on dermatophytes (Lopes et al. 2017; Hameed and Fatima 2019). Table 10.1 shows the values of the minimum inhibitory concentration (MIC) of EOs against dermatophytes. The average MIC values for dermatophytes studied under the effect of citronellol is at least 50% higher than

Table 10.1 Minimal inhibitory concentration of EOs against dermatophytes

Essential oils (main components >18%)	Dermatophytes (MIC)	References
Thymus pulegioides (thymol, carvacrol)	*E. floccosum* FF9 (0.16 μL/mL), *T. rubrum* FF5 (0.32 μL/mL), *T. mentagrophytes* FF7 (0.16 μL/mL), *M. canis* FF1 (0.16 μL/mL), *M.* gypseum FF3 (0.16 μL/mL)	Pinto et al. (2006)
Ocimum gratissimum (thymol)	*T. mentagrophytes* (100 μL/mL), *T. interdigitale* (80 μL/mL), *T. rubrum* (80 μL/mL), *T. erinaceum* (80 μL/mL), *T. soudanense* (80 μL/mL), *T. violaceum* (150 μL/mL), *M. canis* (80 μL/mL), *M. gypseum* (100 μL/mL), *E. flocosum* (150 μL/mL)	Koba et al. (2009)
Cinnamomum zeylanicum (eugenol)	*M. canis* (4.5 μL/mL), *M. gypseum* (7.5 μL/mL), *T. mentagrophytes* (7.5 μL/mL), *T. terrestre* (10 μL/mL), *T. erinacei* (7.5 μL/mL).	Nardoni et al. (2015)
Origanum majorana (carvacrol)	*M. canis* (0.5 μL/mL), *M. gypseum* (2.0 μL/mL), *T. mentagrophytes* (1.0 μL/mL), *T. terrestre* (2.0 μL/mL), *T. erinacei* (1.5 μL/mL)	
Origanum verum (carvacrol)	*M. canis* (0.025 μL/mL), *M. gypseum* (0.025 μL/mL), *T. mentagrophytes* (0.5 μL/mL), *T. terrestre* (0.25 μL/mL), *T. erinacei* (0.5 μL/mL)	
Satureja montana (carvacrol)	*M. canis* (0.5 μL/mL), *M. gypseum* (2.0 μL/mL), *T. mentagrophytes* (2.0 μL/mL), *T. terrestre* (3.0 μL/mL), *T. erinacei* (2.0 μL/mL).	
Thymus serpyllum (thymol)	*M. canis* (0.025 μL/mL), *M. gypseum* (0.025 μL/mL), *T. mentagrophytes* (0.10 μL/mL), *T. terrestre* (0.10 μL/mL), *T. erinacei* (0.20 μL/mL)	
Eucalyptus globulus (1,8-cineole)	*M. canis* (0.1 μL/mL), *M. gypseum* (0.5 μL/mL), *T. mentagrophytes* (0.5 μL/mL), *T. terrestre* (1.5 μL/mL), *T. erinacei* (0.5 μL/mL)	
Thymus capitellatus (1,8-cineole)	*E. floccosum* (0.64 μL/mL), *T. rubrum* (0.64 μL/mL), *T. mentagrophytes* (0.64 μL/mL), *T. canis* (0.64 μL/mL), *M. gypseum* (1.25 μL/mL)	Salgueiro et al. (2006)
Eucalyptus smithii (1,8-cineole)	*M. canis* ATCC 32903 (500 μg/mL), *M. gypseum* ATCC 14683 (1000 μg/mL), *T. mentagrophytes* ATCC 9533 (250 μg/mL), *T. mentagrophytes* ATCC 11481 (125 μg/mL), *T. rubrum* CCT 5507 (62.5 μg/mL)	Baptista et al. (2015)
Lavandula luisieri (1,8-cineole)	*T. rubrum* (MUM 08.12, 08.13, 09.08, 09.25, 09.27, 09.29, 10.128, 10.132), *T. rubrum* ATCC MYA 4438 (All = 200 μg/mL). *T. mentagrophytes* ATCC MYA 4439 (200 μg/mL). *T. interdigitale* (MUM 09.21) (>400 μg/mL)	Dias et al. (2016)
Mentha spicata (carvone)	*E. floccosum* (2 μL/mL), *M. canis* (1 μL/mL), *T. mentagrophytes* (0.75 μL/mL), *T. rubrum* (1 μL/mL)	Ali-Shtayeh et al. (2019)

(continued)

Table 10.1 (continued)

Essential oils (main components >18%)	Dermatophytes (MIC)	References
Cymbopogon citratus (neral, geranial)	*T. rubrum* (MUM 08.12, 08.13, 09.08, 09.25, 09.27, 09.29, 10.128, 10.132), *T. rubrum* ATCC MYA 4438 (All = 200 μg/mL). *T. mentagrophytes* ATCC MYA 4439 (200 μg/mL). *T. interdigitale* (MUM 09.21) (>400 μg/mL)	Dias et al. (2016)
Litsea cubeba (neral, geranial)	*M. canis* (0.025 μL/mL), *M. gypseum* (0.25 μL/mL), *T. mentagrophytes* (0.25 μL/mL), *T. terrestre* (1.5 μL/mL), *T. erinacei* (0.25 μL/mL).	Nardoni et al. (2015)
Illicium verum ((E)-anethole)	*M. canis* (3.0 μL/mL), *M. gypseum* (1.0 μL/mL), *T. mentagrophytes* (0.2 μL/mL), *T. terrestre* (1.5 μL/mL), *T. erinacei* (3.5 μL/mL).	
Foeniculum vulgare ((E)-anethole)	*M. canis* (0.25 μL/mL), *M. gypseum* (0.5 μL/mL), *T. mentagrophytes* (1.5 μL/mL), *T. terrestre* (1.5 μL/mL), *T. erinacei* (3.0 μL/mL)	
Mentha spicata (menthol)	*M. canis* (2.0 μL/mL), *M. gypseum* (3.0 μL/mL), *T. mentagrophytes* (3.0 μL/mL), *T. terrestre* (3.0 μL/mL), *T. erinacei* (3.0 μL/mL)	
Santalum album ((Z)-α-santalol)	*M. canis* (7.5 μL/mL), *M. gypseum* (7.5 μL/mL), *T. mentagrophytes* (7.5 μL/mL), *T. terrestre* (10 μL/mL), *T. erinacei* (7.5 μL/mL)	
Pelargonium graveolens (citronellol)	*M. canis* (0.25 μL/mL), *M. gypseum* (1.5 μL/mL), *T. mentagrophytes* (0.75 μL/mL), *T. terrestre* (0.75 μL/mL), *T. erinacei* (0.75 μL/mL)	
Ocimum basilicum (linalool)	*M. canis* (1.0 μL/mL), *M. gypseum* (3.0 μL/mL), *T. mentagrophytes* (2.5 μL/mL), *T. terrestre* (3.0 μL/mL), *T. erinacei* (2.5 μL/mL)	
Lippia alba (linalool)	*T. rubrum* (39 μg/mL), *M. gypseum* (312 μg/mL), *E. floccosum* (156 μg/mL)	Costa et al. (2014)
Cymbopogon martini (*trans*-geraniol)	*M. gypseum* (200 ppm), *T. rubrum* (150 ppm)	Prasad et al. (2010)
Thymus villosus subsp. lusitanicus (geranyl acetate)	*T. rubrum* CECT 2794 (0.04 μL/mL), *T. mentagrophytes* FF7 (0.16 μL/mL), *T. interdigitale* CECT 2958 (0.16 μL/mL), *T. verrucosum* CECT 2992 (0.64 μL/mL), *M. canis* CECT 2905 (0.16 μL/mL), *E. floccosum* FF9 (0.08 μL/mL)	Pinto et al. (2013b)
Helichrysum italicum (neryl acetate)	*M. canis* (5.0 μL/mL), *M. gypseum* (10 μL/mL), *T. mentagrophytes* (10 μL/mL), *T. terrestre* (10 μL/mL), *T. erinacei* (10 μL/mL)	Nardoni et al. (2015)
Citrus limon (limonene)	*M. canis* (2.5 μL/mL), *M. gypseum* (2.5 μL/mL), *T. mentagrophytes* (5.0 μL/mL), *T. terrestre* (7.5 μL/mL), *T. erinacei* (5.0 μL/mL).	Nardoni et al. (2015)
Citrus medica (limonene)	*M. canis* (4.0 μL/mL), *M. gypseum* (5.0 μL/mL), *T. mentagrophytes* (7.5 μL/mL), *T. terrestre* (10.0 μL/mL), *T. erinacei* (8.0 μL/mL)	

(continued)

Table 10.1 (continued)

Essential oils (main components >18%)	Dermatophytes (MIC)	References
Citrus lemon (limonene)	*M. fulvum* MTCC 2837 (0.9 μg/mL), *T. tonsurans* MTCC 8475 (0.4 μg/mL), *M. canis* MTCC 2820 (1.2 μg/mL), *T. rubrum* MTCC 296 (0.8 μg/mL), *T. mentagrophytes* MTCC 7687 (0.8 μg/mL)	Jain and Sharma 2017
Boswellia sacra (α-thujene)	*M. canis* (5.0 μL/mL), *M. gypseum* (7.5 μL/mL), *T. mentagrophytes* (7.5 μL/mL), *T. terrestre* (10.0 μL / mL), *T. erinacei* (8.0 μL/mL)	Nardoni et al. (2015)
Chenopodium ambrosioides (*m*-cymene)	*M. gypseum* (700 ppm), *T. rubrum* (350 ppm)	Prasad et al. (2010)
Cryptomeria japonica (δ-cadinene)	*T. rubrum* (313 μg/mL)	Takao et al. (2012)
Juniperus communis ssp. alpina (α-pinene). Berries oil	*E. floccosum* FF9 (1.25 μL/mL), *T. rubrum* FF5 (1.25 μL/mL), *T. mentagrophytes* FF7 (1.25 μL/mL), *M. canis* FF1 (1.25 μL/mL), *M. gypseum* FF3 (2.5 μL/mL)	Cavaleiro et al. (2006)
Juniperus oxycedrus ssp. oxycedrus (α-pinene) Berries oil	*E. floccosum* FF9 (0.32 μL/mL), *T. rubrum* FF5 (0.32 μL/mL), *T. mentagrophytes* (0.32 μL/mL), *M. canis* FF1 (0.32 μL/mL), *M. gypseum* F F3 (0.32 μL/mL)	
Juniperus oxycedrus ssp. oxycedrus (α-pinene) Leaves oil	*E. floccosum* FF9 (0.08 μL/mL), *T. rubrum* FF5 (0.08 μL/mL), *T. mentagrophytes* FF7 (0.16 μL/mL), *M. canis* FF1 (0.08 μL/mL), *M. gypseum* FF3 (0.16 μL/mL).	
Juniperus turbinata (α-pinene) Berries oils	*E. floccosum* (0.64 μL/mL), *T. rubrum* FF5 (1.25 μL/mL), *T. mentagrophytes* (1.25 μL/mL), *M. canis* FF1 (0.32 μL/mL), *M. gypseum* FF3 (1.25 μL/mL)	
Juniperus turbinata (α-pinene) Leaves oils	*E. floccosum* (0.64 μL/mL), *T. rubrum* FF5 (0.64 μL/mL), *T. mentagrophytes* (1.25 μL/mL), *M. canis* FF1 (0.64 μL/mL), *M. gypseum* FF3 (1.25 μL/mL)	
Myrtus communis (tricyclene, 1,8-cineole)	*M. canis* (2.0 μL/mL), *M. gypseum* (3.0 μL/mL), *T. mentagrophytes* (1.5 μL/mL), *T. terrestre* (3.0 μL/mL), *T. erinacei* (2.0 μL/mL)	Nardoni et al. (2015)
Rosmarinus officinalis (α-pinene, 1,8-cineole)	*M. canis* (2.5 μL/mL), *M. gypseum* (2.5 μL/mL), *T. mentagrophytes* (5.0 μL/mL), *T. terrestre* (5.0 μL/mL), *T. erinacei* (1.5 μL/mL)	
Vernonia chalybaea (β-caryophyllene, bicyclogermacrene)	*T. rubrum* LABMIC 0201, *T. rubrum* LABMIC 0202, *T. rubrum* LABMIC 0203, *T. rubrum* LABMIC 0204 = (1.25 mg/mL)	Nogueira Sobrinho et al. (2020)
Thapsia villosa (limonene, methyleugenol)	*E. floccosum* FF9 (0.64 μL/mL), *T. rubrum* CECT 2794 (0.64 μL/mL), *T. mentagrophytes* FF7 (0.64 μL/mL), *T. mentagrophytes var. interdigitale* CECT 2958 (1.25 μL/mL), *T. verrucosum* CECT 2992 (1.25 μL/mL), *M. canis* FF1 (0.64 μL/mL), *M. gypseum* CECT 2908 (1.25 μL/mL)	Pinto et al. (2017)

(continued)

Table 10.1 (continued)

Essential oils (main components >18%)	Dermatophytes (MIC)	References
Baccharis trimera (β-pinene, carquejyl acetate)	*T. mentagrophytes* ATCC 9533 (31.25 μg/mL), *T. mentagrophytes* ATCC 11480 (125 μg/mL), *T. rubrum* CCT 5507 (0.03 μg/mL), *M. canis* ATCC 32903 (0.24 μg/mL), *M. gypseum* ATCC 14683 (125 μg/mL)	Caneschi et al. (2015)
Citrus bergamia (linalool acetate, limonene)	*M. canis* (4.0 μL/mL), *M. gypseum* (5.0 μL/mL), *T. mentagrophytes* (5.0 μL/mL), *T. terrestre* (5.0 μL/mL), *T. erinacei* (7.5 μL/mL)	Nardoni et al. (2015)
Angelica major (α-pinene, E-β-ocimene)	*T. mentagrophytes* FF7 (0.32 μL/mL), *T. mentagrophytes var. interdigitale* CECT 2958 (0.64 μL/mL), *T. rubrum* CECT 2794 (0.32 μL/mL), *T. verrucosum* CECT 2992 (1.25 μL/mL), *M. canis* FF1 (0.32 μL/mL), *M. gypseum* CECT 2908 (0.64 μL/mL), *E. floccosum* FF9 (0.32 μL/mL)	Cavaleiro et al. (2015)
Ferulago capillaris (α-pinene, limonene)	*T. mentagrophytes* FF7 (0.64 μL/mL), *M. canis* FF1 (0.32 μL/mL), *T. rubrum* CECT 2794 (0.32 μL/mL), *M. gypseum* CECT 2905 (0.64 μL/mL), *E. floccosum* FF9 (0.64 μL/mL)	Pinto et al. (2013a)

those of geraniol (Shin and Lim 2004; Pereira et al. 2015). The better antidermatophyte capacity of geraniol compared to citronellol could be related to its lower molar volume. Borneol, a secondary bicyclic alcohol, showed higher MIC values than linalool, a tertiary aliphatic alcohol against *Epidermophyton floccosum, T. rubrum,* and *Trichophyton mentagrophytes* (Salgueiro et al. 2006). The difference in the antidermatophytic activity could be related to a higher Log P value of linalool. However, linalool esters lose the antifungal capacity, and their MIC values ranged from 0.32 to 0.64 μL/mL (Salgueiro et al. 2006). The aldehydes α/β unsaturated have a double bond between C_2-C_3, such as neral, geranial, and cinnamaldehyde. The conjugation of the carbonyl group with its α/β unsaturation transforms the C_3 of this molecule into the preferred site for a nucleophilic attack. This strong reactivity allows these molecules to form adducts with DNA, interact with electron-rich proteins, or act as thiol alkylators (Benigni 2005). The percentage of inhibition of *T. rubrum* at a concentration of 0.04% (v/v) is 95% for cinnamaldehyde and 69% for citral (Khan and Ahmad 2011a). The great antifungal activity of cinnamaldehyde with respect to citral is related to the Lowest Unoccupied Molecular Orbital (LUMO) which is an electrophilic descriptor that describes the total ability of a molecule to attack sites rich in electrons and is greater in cinnamaldehyde than in citral (Benigni 2005). The stereoisomers often exert different antifungal effects, with the *trans* isomers showing higher antifungal activity than the *cis* one (Miron et al. 2014). Among monoterpenes with an instilled α/β aldehyde group, geranial that exhibits the C_2-C_3 junction with isomerism (E) shows better anti-dermatophyte activity, that is, four times more active than neral (Miron et al. 2014). Among the primary alcohols with C_2-C_3 unsaturation, the (E) isomer geraniol shows antifungal properties superior to its (Z) isomer nerol (Miron et al. 2014). It is clear that the isomerism of the C_2-C_3 junction in aliphatic monoterpenes plays an important role in its antifungal potential, not so

much the functional group, alcohol, or aldehyde. α-Bisabolol, a sesquiterpenic monocyclic alcohol, shows 30 times more antifungal activity against *T. rubrum* and *T. interdigitale* than the sesquiterpenic acyclic alcohol nerolidol (De Oliveira et al. 2020). This effect could be due to the higher boiling of α-bisabolol point, which results in less loss of the compound in time, favoring the antifungal activity.

Among phenylpropanoids, an increase in the number of rings (comparing monocyclic with bi- and tricyclic) results in a better antifungal activity (Zacchino et al. 1999). Regarding monocyclic phenylpropanoids, the presence of a halogen in C_4 improves its antifungal activity more than ten times compared to ketoconazole (Zacchino et al. 1999).

3 Antifungal Mechanisms of Action of Essential Oils and Their Components

Among synthetic antifungals, allylamines act by inhibiting squalene epoxidase, which prevents the formation of squalene-2,3-epoxide, thus affecting ergosterol biosynthesis. On the other hand, azole drugs exert their effect through the inhibition of the enzyme 14-α-demethylase, which is responsible for the conversion of lanosterol to ergosterol. Likewise, morpholin-based antifungals also avoid ergosterol biosynthesis, by affecting C-14 sterol reductase and C-8 sterol isomerase, different enzymes that are targeted by allylamines and azoles (Hameed and Fatima 2019; Kumari and Singh 2020). Such mechanisms affect the integrity, permeability, and morphology of the fungal plasma membrane (De Oliveira Lima et al. 2017; Poojary 2017; Martínez-Matías and Rodríguez-Medina 2018). As explained before, the antifungal mechanism of an EO is multi-target and will depend to a large extent on the nature of its components and their concentration. However, the interaction with the plasma membrane and the alteration of its permeability could be considered as the first step in the mode of action of any EO (Swamy et al. 2016; Tariq et al. 2019). To determine the effect of EOs or their individual components on the fungal membrane, sorbitol is usually added along with the treatment compound. Sorbitol acts as an osmotic protective agent, managing to stabilize the fungal protoplasm against exogenous stressors. If the EOs or any of their components affect the plasma membrane, sorbitol would act as an osmotic stabilizer. Therefore, the MIC values of the treatment would increase compared to the MIC values in the absence of sorbitol. Experiments that evaluated the anti-dermatophyte properties of citral, geraniol, nerol, cinnamaldehyde, and eugenol in the presence of sorbitol have shown no difference in their MIC values compared to those where the osmotic protection compound is absent (Khan and Ahmad 2011a; Miron et al. 2014). Still, other scientific articles showed effects on the plasma membrane through the action of EOs or monoterpenes (Pereira et al. 2015; Flores et al. 2016; Ali-Shtayeh et al. 2019). Microscopic observations of different species of fungi exposed to EOs or their components clearly showed damage of varying magnitude on the hyphal morphology. The most frequent changes observed are grouping, wrinkling, and compression of

the hyphae, which loses their cylindrical shape. These changes produced by antifungal treatments of EOs, their components, or synthetic drugs are related to the loss of cellular cytoplasm (Baptista et al. 2015; Caneschi et al. 2015). Nerolidolol and, to a lesser extent, α-bisabolol interfere with the functionality of the fungal membrane, causing significant losses of K^+ (De Oliveira et al. 2020). The structural architecture of the fungal cell membrane depends in part on the ergosterol content, which in addition to having a structural role in the formation of the fungal membrane is related to the functional stability of membrane-bound enzymes (Pereira et al. 2015). The mode of action of azole-type heterocyclic antifungals is by blocking the biosynthetic pathway of ergosterol (De Oliveira Lima et al. 2017; Poojary 2017; Martínez-Matías and Rodríguez-Medina 2018). To assess the affinity of antifungal drugs, either synthetic or natural, exogenous ergosterol is added to the culture medium. If the antifungals interact with ergosterol, the MIC of that treatment is increased compared to the control MIC. Through this technique, it was possible to determine that citral, geraniol, and nerol form complexes with ergosterol. This observation leads us to suggest that the interaction of terpenes with ergosterol would be one of the possible mechanisms of antifungal action of EOs or their components (Miron et al. 2014). The morphogenesis of the fungus is one of the main factors in the pathogenesis of dermatophytes, and from its study, we can deduce their growth and infection capacities. The presence of geraniol stimulates the production of chlamydoconidia in *T. rubrum*. This resistance structure represents a defense mechanism that allows the fungus to survive in adverse environmental conditions or in stress caused by toxic agents (Pereira et al. 2015; Hay and Ashbee 2016). Citronellol with the reduced C_2-C_3 bond does not induce the formation of chlamydoconidia; in fact, the configuration (E) of the C_2-C_3 bond in geraniol would be the determinant of this effect (Pereira et al. 2015). During the development of dermatophytes, the presence of microconidia, macroconidia, arthroconidia, pseudophyphae, and true hyphae are usually observed. Microconidia are formed in the conidiophores located in the hyphae, while arthroconodia are formed by fragmentation of the hyphae (Hay and Ashbee 2016; Fajinmi et al. 2019). Adherence of arthroconidia to the host *stratum corneum* is the first step towards contagion and mycelial germination and development (Hay and Ashbee 2016; Jamin et al. 2020). Essential oils can also have a preventive action by inhibiting the formation of conidia (Liu et al. 2009; Flores et al. 2016; Fajinmi et al. 2019). Dermatophytes grow in the keratinized dead tissues, which explains why their development takes place inside the *stratum corneum* of the epidermis, in the keratinized nail bed, or in the nail plate. Within this keratinized environment, the dermatophyte is found as mycelium and arthroconidia; in this parasitic phase, there is no micro or macroconidia (Hay and Ashbee 2016). Due to these characteristics, variations of the effect of the antimycotic synthetic drugs are usually observed. The most vulnerable phase to antifungal products is the mycelial form, while arthroconidial is of greater resistance and is considered the main reason for the failure of many antifungal drugs during clinical treatments of the disease (Khan et al. 2014; Aneke et al. 2020). Arthroconidia is a type of conidia with high resistance to adverse environmental conditions, such as the application of an antifungal drug. In any case, EOs or their components can inhibit their development or

affect them structurally and physiologically (Liu et al. 2009; Khan et al. 2014). *Carum copticum* and *Thymus vulgaris* EOs showed MIC (μg/mL) values of 144 and 72 μg/mL, respectively against *T. rubrum* arthroconidia, while fluconazole, the positive control, had a MIC value of 1600 μg/mL (Khan et al. 2014). Both EOs from *C. copticum* and *T. vulgaris* have p-cymene, thymol, and γ-terpinene as their main components, varying their percentages (Khan et al. 2014). The development of the fungal infection on the host cell will depend on the virulence factors that the fungus secretes into the environment. These secretions are formed by extracellular enzymes, mainly proteinases, among which we can find elastases, keratinases, and gelatinases, and lipases, such as phospholipases and esterases (Khan et al. 2014). The main function of this extracellular fungal complex is to degrade the structural barrier of the skin, nails, or hair and also obtain nutrients. In this way, inhibiting the virulence factors of dermatophytes is a strategy for the development of new antifungals (Khan et al. 2014). Keratin degradation is a process known as sulfitolysis, which involves breaking the disulfide bridges present in keratin (Mercer and Stewart 2019). Many EOs or their pure components are explored as antifungal agents through the evaluation of their protein inhibition capacity, such as keratinases or elastases, and in this way, they can be used to control superficial mycoses by reducing their pathogenicity (Bouyahya et al. 2020). Phenylpropanoids, such as eugenol and cinnamaldehyde, show a 77.9% and 96.6% reduction in elastase activity, respectively, while antikeratinase activity was reduced by 97% by geraniol and 57% by citral (Khan and Ahmad 2011a). *Lippia alba* EO, with a high content of linalool, inhibits the peptidase activities of *E. floccosum* and *T. rubrum* by 25% and 85%, respectively, but was not efficient against the peptidases of *Microsporum gypseum* (Costa et al. 2014). The activity of *T. rubrum* elastases was inhibited 95% by thymol, while *T. vulgaris* EO with 44% of thymol inhibited 90.7% (Khan et al. 2014). The EO of myrrh obtained from *Commiphora molmol* has ruranoeudesma-1,3-diene and menthofuran as its main components. Myrrh EO reduced the elastase activity of dermatophytes by 64% (Mahboubi and Mohammad Taghizadeh Kashani 2016). The EOs from *Ziziphora clinopodioides* and *Ziziphora tenuior* present thymol and p-cymene as the major components, and both inhibited elastase at 0.5 μL/mL in a dose-dependent manner (Mahboubi and Tabar 2018). *Artemisia sieberi* chemotypes with high content of α-thujone and β-thujone showed strong elastase inhibitory activity (>80%) against *M. gypseum, T. rubrum,* and *Microsporum canis* (Mahboubi and Kazempour 2015). *Zataria multiflora* EO has thymol and carvacrol as its main constituents and, at a dose of 1 μL/mL, inhibits 80% of the activity of the pancreatic poricin elastase and 100% of the activity of the elastase produced by dermatophytes (Mahboubi et al. 2017). Another virulent factor is the presence of fungal keratinases in the infection zone. These enzymes were inhibited between 30 and 56% by the EO of *L. alba* (Costa et al. 2014), while the EOs from *T. vulgaris* and *C. copticum* with high content of thymol and γ-terpinene were less effective in reducing the activity of keratinases in *E. floccosum, M. gypseum,* and *T. rubrum* (Khan et al. 2014).

Dermatophytes have developed resistance to azoles. The most important molecular mechanism in the resistance of dermatophytes are changes in target enzymes, changes in membrane permeability, greater efficiency in efflux pumps, and

difficulty in absorbing the antifungal compound (De Oliveira Lima et al. 2017). *Trichophyton* spp. strains when exposed to different anti-dermatophyte compounds produce an increase in mRNA transcription levels for the mdr2 gene, which is related to the protein/ABC transporter complex (ATP-binding cassette). By this mechanism, *Trichophyton* spp. has managed to develop resistance to various antifungal compounds (De Oliveira et al. 2020; Ponte et al. 2020). The EOs and their components are cited in the literature as regulators of efflux pump, the main mechanism of multi-resistance in microorganisms to antifungal drugs (Roy et al. 2012; Limaverde et al. 2017; Martinez-Rossi et al. 2018; Ponte et al. 2020).

A very important problem in the topical treatments of superficial mycoses is the transdermal diffusion of the active ingredient. Nerolidol increases the diffusion for transdermal transport of different bioactive compounds; its aliphatic structure allows its alignment with the lipids of the *stratum corneum* and its passage (Chan et al. 2016). Although the bibliography states that monoterpenes facilitate the transport of active ingredients through the skin (Abdollahi et al. 2020), the transport of itraconazole through the nail was not improved by the use of monoterpenes (Abdollahi et al. 2020). Through QSAR studies it can be seen that terpenic hydrocarbons are the most potent enhancers for transdermal transport, while oxygenated terpenes are the weakest (Ghafourian et al. 2004). However, the high vaporization energy of terpenic hydrocarbons detracts from penetration capacity compared to the lower vaporization energies of alcohols and ketones. A third point is the molecular size, and among alcohols, the smallest size and the largest number of double bonds are indicators of better dermal penetration capacity (Ghafourian et al. 2004).

4 Synergism

Some examples of synergism between the components of sole EOs or EOs combined with synthetic antifungals are seen in Table 10.2. The combination of EOs or their components with conventional antifungal drugs aims to improve the bioactivity of synthetic compounds, minimizing their effective dose, thus reducing possible side effects. The compound β-caryophyllene has the ability to inhibit the growth of different dermatophyte strains and shows synergizing effects when mixed with ketoconazole (Nogueira Sobrinho et al. 2020). Likewise, citronellol and geraniol show strong antifungal synergism with ketoconazole against dermatophytes (Shin and Lim 2004). Nerolidol synergizes with griseofulvin decreasing its MIC value up to eight times, while α-bisabolol shows additive effect with griseofulvin (De Oliveira et al. 2020). Linalool depicts strong synergism with itraconazole and ketoconazole, but does not have any additive or synergistic effect with fluconazole (Ponte et al. 2020). The synergism of linalool with itraconazole and ketoconazole occurs through interference with the activity of ABC proteins/transporters (Ponte et al. 2020). *Otacanthus azureus* EO is characterized by a high concentration of the sesquiterpenes α-humulene and β-copaen-4-α-ol which show strong synergistic antifungal activity with ketoconazole, itraconazole, and fluconazole against *T. mentagrophytes*

(Houël et al. 2014). Ketoconazole combined with *Allium sativum* EO shows a strong synergizing effect while the allicin/ketoconazole combination exerts an additive effect (Pyun and Shin 2006).

Natural products can also be combined seeking to decrease MIC values. Thus, citral, a mixture of geranial and neral, shows greater anti-dermatophyte activity than each of its components separately (Miron et al. 2014). Ramsewak et al. (2003) studied the combination of various monoterpenes and EOs at a concentration of 5 mg/mL each against various dermatophytes, finding a strong synergistic effect in the mixture of camphor, menthol, thymol, and *Eucalyptus citriodora* EO (with α- and

Table 10.2 Minimal inhibitory concentration of monoterpenes and volatile organic compounds against dermatophytes

Monoterpenes	Dermatophytes (MIC)	Synthetic drug	References
β-Caryophyllene	*T. rubrum* LABMIC 0201 (1.25 mg/mL), *T. rubrum* LABMIC 0202 (0.62 mg/mL), *T. rubrum* LABMIC 0203 (1.25 mg/mL), *T. rubrum* LABMIC 0204(1.25 mg/mL)	**Ketoconazole.** *T. rubrum* LABMIC 0201, *T. rubrum* LABMIC 0202, *T. rubrum* LABMIC 0203, *T. rubrum* LABMIC 0204 (1.0 μg/mL)	Nogueira Sobrinho et al. (2020)
Myrcene	*T. mentagrophytes* FF7(5.0 μL/mL), *M. canis* FF1 (1.25 μL/mL), *T. rubrum* CECT 2794 (2.5 μL/mL), *M. gypseum* CECT 2905 (5.0 μL/mL), *E. floccosum* FF9 (1.25 μL/mL)	**Fluconazole**. *T. mentagrophytes* FF7(16 μg/mL), *M. canis* FF1 (128 μg/mL), *T. rubrum* CECT 2794 (16 μg/mL), *M. gypseum* CECT 2905 (128 μg/mL), *E. floccosum* FF9 (16 μg/mL)	Tavares et al. (2010)
Dillapiole	*T. mentagrophytes* FF7(0.08 μL/mL), *M. canis* FF1 (0.08 μL/mL), *T. rubrum* CECT 2794 (0.08 μL/mL), *M. gypseum* CECT 2905 (0.08 μL/mL), *E. floccosum* FF9 (0.08 μL/mL)	**Fluconazole**. *T. mentagrophytes* FF7(16 μg/mL), *M. canis* FF1 (128 μg/mL), *T. rubrum* CECT 2794 (16 μg/mL), *M. gypseum* CECT 2905 (128 μg/mL), *E. floccosum* FF9 (16 μg/mL)	Marongiu et al. (2007)
α-Bisabolol	*T. rubrum* LM4 (16 μg/mL), *T. interdigitale* H6 (16 μg/mL), *T. interdigitale* Dmdr^2 (08 μg/mL)	**Griseofulvin.** *T. rubrum* LM4 (8 μg/mL), *T. interdigitale* H6 (8 μg/mL), *T. interdigitale* Dmdr^2 (8 μg/mL) **Chlorpromazine.** *T. rubrum* LM4 (0.5 μg/mL), *T. interdigitale* H6 (0.5 μg/mL), *T. interdigitale* Δmdr^2 (1 μg/mL)	De Oliveira et al. (2020)
Nerolidol	*T. rubrum* LM4 (512 μg/mL), *T. interdigitale* H6 (512 μg/mL), *T. interdigitale* Δmdr^2 (256 μg/mL)		

(continued)

Table 10.2 (continued)

Monoterpenes	Dermatophytes (MIC)	Synthetic drug	References
Chavicol	*M. canis* (625 µg/mL), *M. gypseum* (625 µg/mL), *T. mentagrophytes* (312.5 µg/mL), *T. rubrum* (312.5 µg/mL), *T. tonsurans* (312.5 µg/mL)		De Castro-Ontengco and Capal (2019)
1,8-cineole	*M. canis* (>2500 µg/mL), *M. gypseum* (>2500 µg/mL), *T. mentagrophytes* (>2500 µg/mL), *T. rubrum* (>2500 µg/mL), *T. tonsurans* (>2500 µg/mL)		
Eugenol	*M. canis* (<156 µg/mL), *M. gypseum* (312.5 µg/mL), *T. mentagrophytes* (<156 µg/mL), *T. rubrum* (<156 µg/mL), *T. tonsurans* (<156 µg/mL)		
Carvone	*E. floccosum* (0.5 µL/mL), *M. canis* (0.63 µL/mL), *T. mentagrophytes* (0.44 µL/mL), *T. rubrum* (0.5 µL/mL)		Ali-Shtayeh et al. (2019).
Methyl eugenol	*E. floccosum* FF9 (0.32 µL/mL), *T. rubrum* CECT 2794 (0.32 µL/mL), *T. mentagrophytes* FF7 (0.32 µL/mL), *T. mentagrophytes var. interdigitale* CECT 2958 (0.32 µL/mL), *T. verrucosum* CECT 2992 (0.32 µL/mL), *M. canis* FF1 (0.32 µL/mL), *M. gypseum* CECT 2908 (0.32 µL/mL)	**Fluconazole.** *E. floccosum* FF9 (16 µg/mL), *T. rubrum* CECT 2794 (16 µg/mL), *T. mentagrophytes* FF7 (16 µg/mL), *T. mentagrophytes var. interdigitale* CECT 2958 (128 µg/mL), *T. verrucosum* CECT 2992 (>128 µg/mL), *M. canis* FF1 (128 µg/mL), *M. gypseum* CECT 2908 (128 µg/mL)	Pinto et al. (2017)
(R)-(+)-limonene	*E. floccosum* FF9 (0.08 µL/mL), *T. rubrum* CECT 2794 (0.08 µL/mL), *T. mentagrophytes* FF7 (0.16 µL/mL), *T. mentagrophytes var. interdigitale* CECT 2958 (0.16 µL/mL), *T. verrucosum* CECT 2992 (0.16 µL/mL), *M. canis* FF1 (0.08 µL/mL), *M. gypseum* CECT 2908 (0.08 µL/mL)		

(continued)

Table 10.2 (continued)

Monoterpenes	Dermatophytes (MIC)	Synthetic drug	References
Anethole	*M. canis* (1%), *M. gypseum* (1%), *T. mentagrophytes* (2.5%), *T. terrestre* (2.5%), *T. erinacei* (2.5%)	**Griseofulvin.** *M. canis* (1 mg/L), *M. gypseum* (40 mg/L), *T. mentagrophytes* (160 mg/L), *T. terrestre* (40 mg/L), *T. erinacei* (2 mg/L). **Terbinafine.** *M. canis* (0.015 mg/L), *M. gypseum* (0.16 mg/L), *T. mentagrophytes* (16 mg/L), *T. terrestre* (0.16 mg/L), *T. erinacei* (0.01 mg/L) **Itraconazole.** *M. canis* (0.12 mg/L), *M. gypseum* (32 mg/L), *T. mentagrophytes* (32 mg/L), *T. terrestre* (0.8 mg/L), *T. erinacei* (0.25 mg/L)	Nardoni et al. (2015)
Carvacrol	*M. canis* (0.1%), M. *gypseum* (0.25%), *T. mentagrophytes* (2.5%), *T. terrestre* (2.5%), *T. erinacei* (2.5%)		
p-Cymene	*M. canis* (>10%), *M. gypseum* (>10%), *T. mentagrophytes* (>10%), *T. terrestre* (>10%), *T. erinacei* (>1 0%)		
1,8-Cineole	*M. canis* (5%), *M. gypseum* (5%), *T. mentagrophytes* (1%), *T. terrestre* (10%), *T. erinacei* (1%)		
Linalool	*M. canis* (1%), *M. gypseum* (1%), *T. mentagrophytes* (2.5%), *T. terrestre* (2.5%), *T. erinacei* (2.5%)		
Menthol	*M. canis* (0.5%), *M. gypseum* (2.5%), *T. mentagrophytes* (1%), *T. terrestre* (1%), *T. erinacei* (1%)		
Menthone	*M. canis* (1%), *M. gypseum* (1%), *T. mentagrophytes* (2.5%), *T. terrestre* (1%), *T. erinacei* (2.5%)		
α-Pinene	*M. canis* (>10%), *M. gypseum* (>10%), *T. mentagrophytes* (>10%), *T. terrestre* (>10%), *T. erinacei* (>10%)		
γ-Terpinene	*M. canis* (>10%), *M. gypseum* (>10%), *T. mentagrophytes* (>10%), *T. terrestre* (>10%), *T. erinacei* (>10%)		
Neral	*M. canis* (0.1%), *M. gypseum* (0.1%), *T. mentagrophytes* (0.25%), *T. terrestre* (0.25%), *T. erinacei* (0.25%)		
Eugenol	*M. canis* (0.1%), *M. gypseum* (0.25%), *T. mentagrophytes* (0.25%), *T. terrestre* (0.25%), *T. erinacei* (0.25%)		
Geraniol	*M. canis* (0.25%), *M. gypseum* (0.25%), *T. mentagrophytes* (1%), *T. terrestre* (0.5%), *T. erinacei* (0.5%)		
Geranial	*M. canis* (0.1%), *M. gypseum* (0.1%), *T. mentagrophytes* (0.25%), *T. terrestre* (1.5%), *T. erinacei* (0.25%)		
Citronellol	*M. canis* (0.25%), *M. gypseum* (0.25%), *T. mentagrophytes* (0.25%), *T. terrestre* (0.5%), *T. erinacei* (0.5%)		
Limonene	*M. canis* (>10%), *M. gypseum* (>10%), *T. mentagrophytes* (>10%), *T. terrestre* (>10%), *T. erinacei* (>10%)		
Thymol	*M. canis* (0.05%), *M. gypseum* (0.25%), *T. mentagrophytes* (0.12%), *T. terrestre* (0.25%), *T. erinacei* (0.25%)		
Fenchone	*M. canis* (0.25%), *M. gypseum* (0.5%), *T. mentagrophytes* (1%), *T. terrestre* (1%), *T. erinacei* (1.5%)		

(continued)

Table 10.2 (continued)

Monoterpenes	Dermatophytes (MIC)	Synthetic drug	References
Geraniol	*T. rubrum* ATCC 1683, LM98, LM130, LM 222, LM 309, LM 333, LM 422, LM 582, LM 600, LM 640, LM 710, LM 713, LM 720, LM 722. (MIC μg/mL = 32, 64, 64, 64, 16, 32,32, 16, 32, 256, 16,16, 64, 64.)		Pereira et al. (2015)
Citronellol	*T. rubrum* ATCC 1683, LM98, LM130, LM 222, LM 309, LM 333, LM 422, LM 582, LM 600, LM 640, LM 710, LM 713, LM 720, LM 722. (MIC μg/mL = 128, 128, 64, 32, 32, 8, 128, 64, 32, 128, 256, 32, 1024, 128.)		
β-Ocimene	*T. mentagrophytes* FF7 (0.64 μL/mL), *T. mentagrophytes var. interdigitale* CECT 2958 (0.32 μL/mL), *T. rubrum* CECT 2794 (0.08 μL/mL), *T. verrucosum* CECT 2992 (0.32 μL/mL), *M. canis* FF1 (0.16 μL/mL), *M. gypseum* CECT 2908 (0.64 μL/mL), *E. floccosum* FF9 (0.64 μL/mL)	**Fluconazole.** *T. mentagrophytes* FF7 (16 μg/mL), *T. mentagrophytes var. interdigitale* CECT 2958 (128 μg/mL), *T. rubrum* CECT 2794 (16 μg/mL), *T. verrucosum* CECT 2992 (>128 μg/mL), *M. canis* FF1 (128 μg/mL), *M. gypseum* CECT 2908 (128 μg/mL), *E. floccosum* FF9 (16 μg/mL)	Cavaleiro et al. (2015)
α-Pinene	*T. mentagrophytes* FF7 (0.32 μL/mL), *T. mentagrophytes var. interdigitale* CECT 2958 (0.32 μL/mL), *T. rubrum* CECT 2794 (0.08 μL/mL), *T. verrucosum* CECT 2992 (0.32 μL/mL), *M. canis* FF1 (0.32 μL/mL), *M. gypseum* CECT 2908 (0.64 μL /mL), *E. floccosum* FF9 (0.32 μL/mL)		
Neral	*T. rubrum* (32 μg/mL), *T. menthagrophytes* (128 μg/mL), M. *canis* (80 μg/mL), *M. gypseum* (>128 μg/mL)	**Terbinafine.** *T. rubrum* (0.25 μg/mL), T. *menthagrophytes* (2.0 μg/mL), *M. canis* (0.25 μg/mL), *M. gypseum* (0.25 μg/mL)	Miron et al. (2014)
Geranial	*T. rubrum* (8.0 μg/mL), *T. menthagrophytes* (32 μg/mL), *M. canis* (48 μg/mL), *M. gypseum* (32 μg/mL)		
Citral	*T. rubrum* (4.0 μg/mL), *T. menthagrophytes* (32 μg/mL), *M. canis* (48 μg/mL), *M. gypseum* (16 μg/mL)		
Nerol	*T. rubrum* (64 μg/mL), *T. menthagrophytes* (128 μg/mL), *M. canis* (128 μg/mL), *M. gypseum* (128 μg/mL)		
Geraniol	*T. rubrum* (64 μg/mL), *T.* menthagrophytes (32 μg/mL), *M. canis* (40 μg/mL), *M. gypseum* (128 μg/mL)		

(continued)

Table 10.2 (continued)

Monoterpenes	Dermatophytes (MIC)	Synthetic drug	References
α-Pinene	*T. mentagrophytes* FF7 (0.32 μL/mL), *M. canis* FF1 (0.16 μL/mL), *T. rubrum* CECT 2794 (0.08 μL/mL), *M. gypseum* CECT 2905 (0.16 μL/mL), *E. floccosum* FF9 (0.16 μL/mL)	**Fluconazole.** *T. mentagrophytes* FF7 (0.32 μg/mL), *M. canis* FF1 (128 μg/mL), *T. rubrum* CECT 2794 (16 μg/mL), *M. gypseum* CECT 2905 (128 μg/mL), *E. floccosum* FF9 (16 μg/mL)	Pinto et al. (2013a)
Limonene	*T. mentagrophytes* FF7 (2.5 μL/mL), *M. canis* FF1 (0.64 μL/mL), *T. rubrum* CECT 2794 (0.64 μL/mL), *M. gypseum* CECT 2905 (1.25 μL/mL), *E. floccosum* FF9 (1.25 μL/mL)		
Geranyl acetate	*T. rubrum* CECT 2794 (0.32 μL/mL), *T. mentagrophytes* FF7 (0.32 μL/mL), *T. interdigitale* CECT 2958 (0.32 μL/mL), *T. verrucosum* CECT 2992 (0.64 μL/mL), *M. canis* CECT 2905 (0.16 μL/mL), *E. floccosum* FF9 (0.16 μL/mL)	**Fluconazole.** *T. rubrum* CECT 2794 (16 μg/mL), *T. mentagrophytes* FF7 (16 μg/mL), *T. interdigitale* CECT 2958 (128 μg/mL), *T. verrucosum* CECT 2992 (>128 μg/mL), *M. canis* CECT 2905 (128 μg/mL), *E. floccosum* FF9 (16 μg/mL)	Pinto et al. (2013b)
Terpinen-4-ol	*T. rubrum* CECT 2794 (1.25 μL/mL), *T. mentagrophytes* FF7 (2.5 μL/mL), *T. interdigitale* CECT 2958 (1.25 μL/mL), *T. verrucosum* CECT 2992 (1.25 μL/mL), *M. canis* CECT 2905 (1.25 μL/mL), *M. gypseum* CECT 2905 (2.5 μL/mL), *E. floccosum* FF9 (1.25 μL/mL)		
Linalool	*T. rubrum* CECT 2794 (1.25 μL/mL), *T. mentagrophytes* FF7 (1.25 μL/mL), *T. interdigitale* CECT 2958 (2.5 μL/mL), *T. verrucosum* CECT 2992 (1.254 μL/mL), *M. canis* CECT 2905 (2.5 μL/mL), *M. gypseum* CECT 2905 (1.25 μL/mL), *E. floccosum* FF9 (1.25 μL/mL)		
Geraniol	*T. rubrum* CECT 2794 (0.16 μL/mL), *T. mentagrophytes* FF7 (0.08 μL/mL), *T. interdigitale* CECT 2958 (0.16 μL/mL), *T. verrucosum* CECT 2992 (0.16 μL/mL), *M. canis* CECT 2905 (0.16 μL/mL), *M. gypseum* CECT 2905 (0.32 μL/mL), *E. floccosum* FF9 (0.16 μL/mL)		
δ-Cadinene	*T. rubrum* (500 μg/mL)		Takao et al. (2012)
Epi-cubenol	*T. rubrum* (250 μg/mL)		
β-Eudesmol	*T. rubrum* (1000 μg/mL)		

(continued)

Table 10.2 (continued)

Monoterpenes	Dermatophytes (MIC)	Synthetic drug	References
Thymol	*T. mentagrophytes* (50 µL/mL), *T. interdigitale* (100 µL/mL), *T. rubrum* (50 µL/mL), *T. erinaceum* (50 µL/mL), *T. soudanense* (40 µL/mL), *T. violaceum* (30 µL/mL), *M. canis* (50 µL/mL), *M. gypseum* (50 µL/mL), *E. flocosum* (50 µL /mL)		Koba et al. (2009)
γ-Terpinene	*T. mentagrophytes*, *T. interdigitale, T. rubrum, T. erinaceum, T. soudanense, T. violaceum, M. canis, M. gypseum, E. flocosum.* All MIC >500 µL/mL		
p-Cymene	*T. mentagrophytes, T. interdigitale, T. rubrum, T. erinaceum, T. soudanense, T. violaceum, M. canis, M. gypseum, E. flocosum*. All MIC >500 µL/mL		
Carvacrol	*E. floccosum* FF9 (0.08 µL/mL), *T. rubrum* FF5 (0.08 µL/mL), *T. mentagrophytes* FF7 (0.04 µL/mL), *M. canis* FF1 (0.04 µL/mL), *M. gypseum* FF3 (0.04 µL/mL)	**Fluconazole.** *E. floccosum* FF9 (0.16 µg/mL), *T. rubrum* FF5 (0.32 µg/mL), *T. mentagrophytes* FF7 (0.32 µg/mL), *M. canis* FF1 (128 µg/mL), *M. gypseum* FF3 (>128 µg/mL)	Pinto et al. (2006)
p-Cymene	*E. floccosum* FF9 (5.0 µL/mL), *T. rubrum* FF5 (1.25 µL/mL), *T. mentagrophytes* FF7 (5.0 µL/mL), *M. canis* FF1 (2.5 µL/mL), *M. gypseum* FF3 (10 µL/mL)		
γ-Terpinene	*E. floccosum* FF9 (2.56 µL/mL), *T. rubrum* FF5 (5.0 µL/mL), *T. mentagrophytes* FF7 (10.0 µL/mL), *M. canis* FF1 (5.0 µL/mL), *M. gypseum* FF3 (10.0 µL/mL)		
Thymol	*E. floccosum* FF9 (0.16 µL/mL), *T. rubrum* FF5 (0.16 µL/mL), *T. mentagrophytes* FF7 (0.16 µL/mL), *M. canis* FF1 (0.08 µL/mL), *M. gypseum* FF3 (0.16 µL/mL)		
α-Pinene	*E. floccosum* FF9 (0.16 µL/mL), *T. rubrum* FF5 (0.16 µL/mL), *T. mentagrophytes* FF7 (0.32 µL/mL), *M. canis* FF1 (0. µL/mL), *M. gypseum* FF3 (0.32 µL/mL)	**Fluconazole.** *E. floccosum* FF9, *T. rubrum* FF5, *T. mentagrophytes* FF7 (16 µg/mL), *M. canis* FF1, *M. gypseum* FF3 (>128 µg/mL)	Cavaleiro et al. (2006)
δ-3-Carene	*E. floccosum* FF9 (0.16 µL/mL), *T. rubrum* FF5 (0.16 µL/mL), *T. mentagrophytes* FF7 (0.32 µL/mL), *M. canis* FF1 (0.16 µL/mL), *M. gypseum* FF3 (0.64 µL/mL)		

(continued)

Table 10.2 (continued)

Monoterpenes	Dermatophytes (MIC)	Synthetic drug	References
1.8-Cineole	*E. floccosum* (5.0 µL/mL), *T. rubrum* (2.5 µL/mL), *T. mentagrophytes* (5.0 µL/mL), *T. canis* (5.0 µL/mL), *M. gypseum* (10.0 µL/mL)	**Fluconazole.** *E. floccosum* (16 µg/mL), *T. rubrum* (16 µg/mL), *T. mentagrophytes* (16 µg/mL), *T. canis* (128 µg/mL), *M. gypseum* (>128 µg/mL)	Salgueiro et al. (2006)
Borneol	*E. floccosum* (2.5 µL/mL), *T. rubrum* (2.5 µL/mL), *T. mentagrophytes* (2.5 µL/mL), *T. canis* (2.5 µL/mL), *M. gypseum* (2.5 µL/mL)		
Linalool	*E. floccosum* (1.25.0 µL/mL), *T. rubrum* (1.25 µL/mL), *T. mentagrophytes* (1.25 µL/mL), *T. canis* (2.5 µL/mL), *M. gypseum* (2.5 µL/mL)		
Linalyl acetate	*E. floccosum* (0.32 µL/mL), *T. rubrum* (0.32 µL/mL), *T. mentagrophytes* (0.64 µL/mL), *T. canis* (0.32 µL/mL), *M. gypseum* (0.64 µL/mL)		
Benzoic acid	*T. erinacei* (<0.12 mg/mL), *T. mentagrophytes* (0.25 mg/mL), *T. rubrum* (0.25 mg/mL), *T. schoenleinii* (<0.25 mg/mL), *T. soudanense* (0.25 mg/mL), *T. tonsurans* (0.25 mg/mL)		Shin and Lim (2004)
Citronellol	*T. erinacei* (0.5 mg/mL), *T. mentagrophytes* (1.0 mg/mL), *T. rubrum* (2.0 mg/mL), *T. schoenleinii* (1.0 mg/mL), *T. soudanense* (0.50 mg/mL), *T. tonsurans* (2.0 mg/mL)		
Geraniol	*T. erinacei* (0.5 mg/mL), *T. mentagrophytes* (0.5 mg/mL), *T. rubrum* (1.0 mg/mL), *T. schoenleinii* (0.5 mg/mL), *T. soudanense* (0.25 mg/mL), *T. tonsurans* (0.5 mg/mL)		
Thymol	*T. erinacei* (0.25 mg/mL), *T. mentagrophytes* (0.5 mg/mL), *T. rubrum* (0.5 mg/mL), *T. schoenleinii* (0.5 mg/mL), *T. soudanense* (0.5 mg/mL), *T. tonsurans* (0.5 mg/mL)		
Estragole	*T. mucoides* (5 mg/mL), *T. tonsurans* (10 mg/mL)	**Ketoconazole.** *T. mucoides* (12.5 µg/mL), *T. tonsurans* (25 µg/mL)	Shin and Kang (2003)

(continued)

β-pinenes as main components). The combination of *A. sativum* EO (dially sulfide, 57.1%)/*Cymbopogon martinii* EO (geraniol, 80.7%) showed MIC values lower than 0.06 mg/mL against *T. mentagrophytes* while the combination of *Pinus sylvestris* EO (monoterpenic hydrocarbons 79%)/*Origanum vulgare* EO (carvacrol/thymol 74%) showed an MIC of 0.13 mg/mL (Orchard et al. 2019). Other mixtures using *Santalum austrocaledonicum* EO (main component α-santolol) also showed to have an anti-dermatophyte potential (Orchard et al. 2019). On the other hand, many other combinations of EOs were reported to have no effect or simply were antagonistic (Prasad et al. 2010; Orchard et al. 2019).

The great potential of EOs or their components as antifungal agents has led to evaluation of Vicks VapoRub™ (a cream with a formulation based on monoterpenes and phenylpropanoids used for respiratory treatments) as an anti-dermatophyte product, showing encouraging results (Derby et al. 2011), even in immunosuppressed patients (Snell et al. 2016). These results have increased the available commercial topical formulations bearing EOs as bioactive components or as synergizing agents of synthetic antifungal drugs for the treatment of onychomycosis.

5 *Candida*

Candida spp is among the major causative agents of fungal diseases, with approximately 700,000 new cases of invasive candidiasis being reported each year (Góralska et al. 2018; Silva et al. 2019). Relatively few species are pathogens, with most of the *Candida* infections (> 90%) being caused mainly by *C. albicans*, *C. glabrata, C. parapsilosis*, *C. krusei,* and *C. tropicalis* (Köhler et al. 2017; Boral et al. 2018; Góralska et al. 2018). They are commensal microorganisms of skin and mucosal surfaces such as oral cavity, gastrointestinal tract, and urogenital tract, but become opportunistic pathogens in the presence of medical vulnerabilities such as the decline of the host's immune defenses, systemic diseases, extensive wounds (burns and operations), poor oral and dental hygiene, smoking habits, and trauma (Borchers et al. 2017; Peixoto et al. 2017; Góralska et al. 2018; Tedila et al. 2019; Singhi and Saini 2019).

Candida albicans is able to switch its phenotype according to the host environment and stress conditions, and the different morphologies are associated with its metabolism, pathogenesis, biofilm formation ability, and drug resistance (Köhler et al. 2017; Boral et al. 2018; Tedila et al. 2019). Furthermore, *C. albicans* secretes acid proteinases, phospholipases, and lipases that also contribute to its pathogenesis. These enzymes degrade the epithelial cells, and the hyphal extension produce damage by physical force (Köhler et al. 2017; Boral et al. 2018). Hence, species of the genus *Candida* can establish and form biofilms on ocular, oral, intestinal, and vaginal epithelial tissues (Sharifzadeh and Shokri 2016; Peixoto et al. 2017; Boral et al. 2018; Geddes-McAlister and Shapiro 2019; Song and Lee 2019). In addition, they are found forming biofilms in catheters producing invasive fungal infections

associated with high morbidity and mortality. The structure of the mature biofilm consists of yeast and hyphal and pseudohyphal elements immersed in a polysaccharide/protein matrix. The yeast form is essential for biofilm formation since biofilms that contain only hyphae can be easily disrupted (Manoharan et al. 2017; Boral et al. 2018). Biofilms are more resistant to antimicrobial agents and insensitive to host immune responses (Köhler et al. 2017; Manoharan et al. 2017). The increase of fungal infections is related to immunosuppression due to diseases like HIV/AIDS and cancer; pervasive dysbiosis of the human microbiome associated with exposure to broad-spectrum antimicrobials; the use of modern medical devices such as ventilators, stents, and catheters (Geddes-McAlister and Shapiro 2019). *Candida albicans* is the most frequently isolated species from patients with candidiasis (>50%), but infections caused by other non-albicans *Candida* are becoming more significant in different regions of the world (Allen et al. 2015; Góralska et al. 2018; Silva et al. 2019). This is of great relevance since many non-*albicans Candida* species presented intrinsic resistance to fluconazole (Silva et al. 2019). For instance, the 10–15% of the *C. glabrata* clinical isolates are resistant to fluconazole and voriconazole, and this species also present high frequency of multidrug resistance which reduced susceptibility to both azoles and echinocandins (Geddes-McAlister and Shapiro 2019). Besides, the recent isolation of new strains such as *C. auris* with high rates of antifungal drug resistance is a new challenge to human health (Geddes-McAlister and Shapiro 2019). Therefore, new therapies are needed to successfully face these emerging fungal diseases, and the natural products as EOs could be an option due to their previously described properties.

5.1 Essential Oils and Their Components Against Candida *sp.*

The antimicrobial activity is associated with the chemical composition of the EO which depends on many factors, as mentioned above, and generally an EO with high concentrations of monoterpenic and sesquiterpene constituents is more effective as anticandidal agent (Bona et al. 2016; De Toledo et al. 2016; Benzaid et al. 2019; Dutta et al. 2020; El Mokni et al. 2020). Table 10.3 shows the MIC values of different EOs on *C. albicans*, *C. parapsilosis*, *C. glabrata*, and *C. krusei*. Although the literature does not present a standard MIC values (sensitive and resistant) for natural products against *Candida* spp., it was stated that values equal to or lower than 1000 μg/mL confirm sensitivity (De Toledo et al. 2016; Peixoto et al. 2017). Moreover, the EO activity is attributed to the synergic combination of its major and minor compounds, and some investigations found that an EO had better antifungal activity than their individual components. For instance, the major components such as citronellal, p-cymene, and thymol show lower effect on fungal growth compared with the EO (De Toledo et al. 2016; Dutta et al. 2020). In other study, thymol and the EO showed almost the same effects on strains of *C. albicans* and *C. krusei* (Mehriardestani et al. 2020). This discrepancy could be due to the different strains evaluated and the different methodologies used. The bibliography reports that

Table 10.3 Minimal inhibitory concentrations of EOs against *Candida* species

Plant EO	*C. albicans*	*C. glabrata*	*C. parapsilosis*	*C. tropicalis*	References
	MIC μg/mL				
Pelargonium graveolens	500	500	500	250	Essid et al. (2017)
	500	500	–	–	
	500	–	–	–	
	1000	–	–	–	
	1000	–	–	–	
Cinnamomum verum	625	62.5	62.5	312.5	
	312.5	62.5	–	–	
	625	–	–	–	
	625	–	–	–	
	625	–	–	–	
Thymus capitatus	125	125	125	125	
	125	125	–	–	
	125	–	–	–	
	125	–	–	–	
	125	–	–	–	
Syzygium aromaticum	250	250	250	250	
	125	250	–	–	
	250	–	–	–	
	250	–	–	–	
	250	–	–	–	
Cymbopogon nardus	1000	500	500	500	De Toledo et al. (2016)
	1000	500	1000	>1000	
	1000	500	–	1000	
	1000	1000	–	>1000	
Trachyspermum ammi	500	–	–	–	Mehriardestani et al. (2020)
	27	–	–	122.4	Dutta et al. (2020)
	22.4	–	–	127	
	13.8	–	–	–	
	24.2	–	–	–	
Lavandula maroccana	4604	4604	4604	–	Soulaimani et al. (2019)
Lippia lasiocalycina	512	–	–	–	De Almeida et al. (2018)
Senecio anteuphorbium	1024	2048	2048	–	Elhidar et al. (2019)
	2048	–	–	–	
Plectranthus glandulosus	5000	–	–	–	Ngo-Mback et al. (2019)
Aeollanthus heliotropioides	1250	–	–	–	

(continued)

Table 10.3 (continued)

Plant EO	*C. albicans*	*C. glabrata*	*C. parapsilosis*	*C. tropicalis*	References
	MIC μg/mL				
Bubonium imbricatum	750	370	180	–	Aghraz et al. (2016)
Mentha arvensis	2000	–	2000	–	Busato de Feiria et al. (2016)
Mentha piperita	2000	–	500	–	
Laurus nobilis	–	–	0.8	–	Córdoba et al. (2019)
	250	5000	–	500	Peixoto et al. (2017)
	250	–	–	250	
	–	–	0.8	–	Córdoba et al. (2019)
Thymus vulgaris	–	–	0.8	–	
Cymbopogon citratus	–	–	50	–	
Lippia junelliana	–	–	1.6	–	
Calamintha officinalis	–	–	200	–	
Ballota nigra subsp. *uncinata*	625	–	–	–	El Mokni et al. (2020)
Ballota bullata	625	–	–	–	
Foeniculum vulgare	25	–	–	–	Ilić et al. (2019)
Plantago afra	312.5	–	–	–	Hammami et al. (2020)
Eucalyptus globulus	219	219	–	885	Quatrin et al. (2017)

species of *Candida* are more sensitive to different EOs than traditionally used drugs. The EOs from *Ocimum basilicum*, *Lavandula* spp., *Melaleuca alternifolia*, *Satureja montana*, and *Thymus capitatus* inhibited both growth and metabolic activity of *C. albicans* isolated from vaginal swab (resistant to three main azole antifungal drugs) more efficiently than clotrimazole, being *T. capitatus* EO the most effective (Bona et al. 2016). Also, *Cymbopogon nardus* EO was effective against almost all the *Candida* species tested including strains resistant to fluconazole and amphotericin-B, with MIC values that ranged from 250 to 1000 μg/mL (De Toledo et al. 2016). The continuous raising of antibiotic-resistant and less susceptible strains of pathogens to the widely prescribed antifungal drugs is a global public health concern. The EOs could act synergistically with currently used drugs, thus EOs and/or their components is an alternative strategy for the development of new antifungal agents. *Pelargonium graveolens, Cinnamomum verum, Piper mikanianum, Lavandula maroccana, Senecio anteuphorbium*, and *Vanillosmopsis arborea* EOs showed synergistic effect when combined with fluconazole against *Candida* strains (Essid et al. 2017; Rodrigues et al. 2018; Elhidar et al. 2019; Soulaimani et al. 2019; Carneiro et al. 2020), whereas the combination of *L. maroccana* EO with

amphotericin B showed a total synergism against *C. krusei* and a partial synergism against *C. albicans*, *C. glabrata*, and *C. parapsilosis* (Soulaimani et al. 2019).

In general, antifungal agents that are effective against planktonic cell are often ineffective on biofilm, and the incomplete removal of biofilm may result in drug resistance or reinfection (Manoharan et al. 2017). The EOs also inhibit the morphological transition capacity of yeast to hypha, which is one of the greatest virulence factors of *Candida*. The EOs from *C. nardus, Mentha x piperita, P. mikanianum, Aeollanthus heliotropioides,* and *Plectranthus glandulosus* inhibit the formation of filamentous structures, contributing to the controlling of *Candida* infection (De Toledo et al. 2016; Benzaid et al. 2019; Ngo-Mback et al. 2019; Carneiro et al. 2020). The control and elimination of fungal biofilm is difficult, due to several types of molecular, structural, and specifically physiological interactions. In the biofilm, the fungi are more resistant to antimicrobial agents due to the barrier function of the extracellular matrix which contains polysaccharides, proteins, and nucleic acids (Song and Lee 2019). *Mentha piperita* EO decreases biofilm formation of *C. albicans* at concentrations of 10 μL/mL and disrupts mature biofilm (Benzaid et al. 2019). The EOs of cascarilla bark (*Croton eluteria*), helichrysum, coriander, lemon eucalyptus, lemongrass, and lime at 0.01% reduced the formation of biofilm in *C. albicans* by more than 90% and also biofilm thickness (Manoharan et al. 2017). Moreover, *Laurus nobilis* EO reduced the formation of mature biofilm and inhibited the initial adhesion and biofilm formation at concentrations of 1000 μg/mL of *C. albicans* (Peixoto et al. 2017). At concentrations of 2000 μg/mL EO, three *Mentha* species inhibited biofilm formation (90%) and produced disruption of the *C. albicans* biofilm (80%) (Busato de Feiria et al. 2016).

5.2 Mechanisms of Action of Essential Oils in Candida *sp.*

The mechanism of action of EOs is frequently related to the cell wall biosynthesis and the ionic permeability of the cell membrane. Damage of the cell wall and membranes of the yeast was observed in *Candida* cells treated with EO from *T. capitatus*, *L. nobilis,* and *O. basilicum* (Bona et al. 2016; Peixoto et al. 2017; Miao et al. 2020). The first action of phenols compounds present in EOs is a nonspecific interaction with the mitochondrial or plasma membrane. Then, the alkylphenols with isopropyl substituents could be biotransformed into quinones methylene (highly toxic) during the metabolic processes. These could explain the different anticandidal activities observed among phenol compounds (Gallucci et al. 2014). *Pelargonium graveolens* EO acts decreasing the levels of major lipids (palmitic acid, stearic acid, and oleic acid) by 54.71% in cells of *C. albicans*, inhibiting the formation of long chain fatty acids. Consequently, the permeability barrier of the cell wall is disturbed, mainly oleic acid and fatty acid homeostasis. On the other hand, *C. verum* EO strongly reduces the ergosterol biosynthesis (83%) and was, indeed, more effective than fluconazole. The synergic effect of the combination of *C. verum* with fluconazole produced a total inhibition of ergosterol production causing a membrane damaging effect that could increase cell permeability and finally cell death (69.51% of killing rate) (Essid et al. 2017). The secreted aspartic proteases (SAPs) are produced during

the infection, facilitating the penetration of pathogenic *Candida* cells into the host organism. SAPs include 10 proteolytic enzymes that have the capacity to degrade immunoproteins, collagen, and fibronectin. Hence, the use of aspartyl proteinase inhibitors may in fact reduce the yeast virulence and pathogenesis (Köhler et al. 2017; Essid et al. 2017; Benzaid et al. 2019). *Pelargonium graveolens* and *C. verum* EO in combination with fluconazole inhibited 78.31% and 64.72% of SAPs activity, respectively (Essid et al. 2017), while *M. piperita* EO downregulated the expression of genes involved in SAP1, 2, 3, 9, synthesis, which may lead to reducing *C. albicans* virulence. Other mechanisms of this EO was a decreased expression of HWP1, a hyphal-specific adhesion gene that encodes the hyphal cell wall protein promoting *C. albicans* adhesion to different surface (Benzaid et al. 2019). Metabolomics analyses revealed that 34 metabolites changed their abundance in *C. albicans* cells under the sub lethal effect of *O. basilicum* EO. These metabolites were intermediates involved in the carbon, amino acids, polyamines, lipids and fatty acid metabolism, such as glycolysis/gluconeogenesis, pentose phosphate pathway, and TCA cycle, among others (Miao et al. 2020). In future research, metabolomic analyses could help to elucidate with greater detail the mechanisms of actions of EOs.

6 Cryptococcosis

Cryptococcus spp are encapsulated yeasts (3.5–8 μm) that reproduce by single budding, forming a narrow neck between the mother and daughter cells. Exceptionally, multiple budding, elongated shapes, and pseudohyphae are observed. These yeasts have a worldwide distribution (Heitman et al. 2011) and is frequently isolated from soil contaminated with pigeon or other bird droppings. It can be also isolated from dry avian manure accumulated in buildings but not from fresh manure. Birds do not suffer from *C. neoformans* infection but serve as vectors, because of their high body temperature which is approximately 42 °C, conditions that allow the microorganism to survive but not to develop (Vázquez Tsuji et al. 2005). The presence of nitrogenous products, humidity, and alkalinity permits the encapsulated yeast to remain viable for around two years in beard droppings. Additionally, *C. neoformans* is isolated from fruit and vegetables (Jorgensen et al. 2015). The genus *Cryptococcus* comprises many species. Different strains of *C. neoformans* have been grouped into two varieties that include three serotypes depending on their capsular structure: *C. neoformans* var. *neoformans*, A or D serotypes, which are isolated from bird droppings while *C. neoformans* var. *gattii* was isolated from the waste that surrounds the species *Eucalyptus camaldulensis* and *Eucalyptus tereticornis* and belong to B serotype (Heitman et al. 2011; Bandalizadeh et al. 2020).

The infection is acquired by inhalation of the dried yeasts, which easily reaches the alveolar spaces. The person-to-person transmission does not exist, but it has been reported that *Cryptococcus* can be transmitted through transplanted organs. No evidence of direct transmission from animals to humans has been reported (Vázquez Tsuji et al. 2005). The clinical course of cryptococcosis in a patient depends of the

inoculum quantity, the virulence of the infecting strain, and the immunological status of the patient (Gullo et al. 2013). The presence of *Cryptococcus* sp. in the pulmonary alveoli triggers a cellular and humoral immunity response of the host, which under normal conditions control the infection. Due to the increase of immunocompromised patients in the general population, *Cryptococcus* spp. have become habitual opportunistic agents. The clinical manifestation can be varied, from an asymptomatic colonization of the airways to a disseminated infection with a predilection for the central nervous system (Messina et al. 2015). Due to the increase of immunocompromised individuals that develop opportunistic infections of *C. neoformans* and the appearance of resistant strains, the development of safer treatments for the control of this emergent disease becomes necessary. Related to this, antifungal products from phytochemicals could be considered an alternative for *Cryptococcus* spp. control. In this context, EOs could be an alternative of safer treatments. Table 10.4 reports the MIC values and the main composition of EOs from aromatic plants against *C. neoformans*. Abu-Darwish et al. (2015) showed the anti-*Cryptococcus* activity (MIC = 0.64 mg/mL) of *Artemisia herba-alba* EO rich in oxygenated monoterpenes (86.8%), being β-thujone, 1,8-cineole, α-thujone, and camphor their main components. Moreover, the antifungal activity against *C. neoformans* (MIC = 0.11 mg/mL) of *T. vulgaris* was reported by Kamdem et al. (2015), with thymol as the main component (57.9%). Many authors have attributed the antifungal activity of some EOs to the presence of this monoterpene (Bektas et al. 2016; Scalas et al. 2018; Pizzolitto et al. 2020). Khoury et al. (2018) evaluated the chemical compositions and the effect of four EOs from different species belonging to Apiaceae on *C. neoformans*. The EOs from *Ferula elaeochytris* and *Prangos asperula* were mainly composed of monoterpene hydrocarbons (80.2% and 74.4%, respectively), being α-pinene (71.8%) and β-pinene (6.80%) the prevalent compounds of *F. elaeochytris* EO, and α-pinene (9.8%), sabinene (29.8%), α-phellandrene (8.0%), β-phellandrene (19.2%), and nerolidol (9.2%) the major constituents of *P. asperula* EO. A high content of sesquiterpenes (73.1%) was reported for *Smyrnium olusatrum* EO. Moreover, *Daucus carota* EO was rich in hydrocarbons such as α-pinene (27.4%), myrcene (5.3%), α-humulene (9.8%), and D-germacrene (7.0%). The MIC values were 0.13 mg/mL for *F. elaeochytris*, *S. olusatrum*, and *D. carota* EOs, and 0.26 mg/mL for *P. asperula* EO. Lawson et al. (2019) attributed the antifungal effect of *Helianthus* spp. against *C. neoformans* to the dominance of pinene in EO composition. Sesquiterpenes have been suggested to be responsible for the observed anti-*Cryptococcus* activity (MIC = 0.27 mg/mL) of *Plectranthus* spp. EOs (Mothana et al. 2018). Do Prado et al. (2018) reported a high activity against *C. neoformans* (MIC = 0.65 mg/mL) of *Schinus molle* EO, which is characterized by the dominance of pinene (43%) and myrcene (11.5%). Moreover, as shown in Table 10.4, *Zanthoxylum monogynum* EO reported citronellal (9.6%), citronellol (43.3%), and farnesol (32%) as the major compounds of the EO, with significant antifungal effect on *C. neoformans* development (Da Silva et al. 2017). Dos Santos et al. (2015) determined monoterpenes (70.11%) and sesquiterpenes (27.24%) as the major constituents of *Plectranthus amboinicus,* a strong anti-Cryptococcus EO (MIC = 0.01 mg/mL). With regard to *L. nobilis* EO, Sousa

Table 10.4 Minimal inhibitory concentration of EOs against *Cryptococcus neoformans*

PLANT EO	Main components (%)	MIC	References
Artemisia herba-alba	β-Thujone (25.1), α-Thujone (22.9), 1,8-Cineole (20.1)	0.64 mg/mL	Abu-Darwish et al. (2015)
Daucus carota subsp. *maximus*	α-Pinene (27.4), Carotol (26.3), α-Humulene (9.8), D-germacrene (7.0)	0.13 mg/mL	Khoury et al. (2018)
Ferula elaeochytris	α-Pinene (71.8), β-Pinene (6.8)	0.13 mg/mL	
Prangos asperula	Sabinene (29.8), β-Phellandrene (19.2), α-Pinene (9.8), Nerolidol (9.2)	0.26 mg/mL	
Smyrnium olusatrum	Curzerene (31.5), Furanoeremophil-1-one (28.5), Furanodiene (13.1)	0.13 mg/mL	
Apium graveolens	Limonene (50.7), Myrcene (12.5)	4.37 mg/mL	Kamdem et al. (2015)
Thymus vulgaris	Thymol (57.9), p-Cymene (10.3), Linalool (6.9)	0.11 mg/mL	
Plectranthus cylindraceus	Maaliol (42.8), Camphor (7.2)	0.27 mg/mL	Mothana et al. (2018)
Plectranthus asirensis	β-Caryophyllene (13.3), Spathulenol (8.7), Bicyclogermacrene (7.4),	0.27 mg/mL	
Plectranthus barbatus	Borneol (20.7)	0.27 mg/mL	
Helianthus annuus "Chianti"	α-Pinene (50.6), Camphene (7.3), Limonene (7.2), Bornyl acetate (7.1)	0.08 mg/mL	Lawson et al. (2019)
Helianthus annuus "Mammoth"	α-Pinene (48.9), Sabinene (17.0), Limonene (7.1)	0.15 mg/mL	
Helianthus strumosus	α-Pinene (58.6), Myrcene (9.8)	0.08 mg/mL	
Plectranthus amboinicus	Carvacrol (37.7), γ-Terpinene (14.7), (Z)-Caryophyllene (14.1), p-Cymene (12.0), Trans-α-bergamotene (8.2)	0.01 mg/mL	Dos Santos et al. (2015)
Laurus nobilis	Isoeugenol (57.0), Myrcene (15.9), Chavicol (9.3)	0.26 mg/mL	Sousa Pinheiro et al. (2017)
Artemisia stricta	Capillene (41.6), Spathulenol (14.6), β- Caryophyllene (13.4)	5.00 mg/mL	Manika et al. (2016)
Baccharis parvidentata	Sabinene (15.2), Himachalol (10.3), α-Pinene (9.2)	1.25 mg/mL	Perera et al. (2016)
Lippia origanoides	(*E*)-Methyl cinnamate (40.0), Hedycaryol (8.0), β-Eudesmol (7.3), α-Eudesmol (7.6)	0.08 mg/mL	
Zanthoxylum monogynum	Farnesol (32.0), Citronellol (43.3), Citronellal (9.6)	1.50 mg/mL	Da Silva et al. (2017)
Schinus molle	β-Pinene (25.2), Epi-α-cadinol (21.3), α-Pinene (18.7)	0.65 mg/mL	Do Prado et al. (2018)

Pinheiro et al. (2017) reported a MIC value of 0.26 mg/mL being the isoeugenol (57.0%) and myrcene (15. %) the main constituents. *Artemisia stricta* EO reported an effect against *C. neoformars* where capillene, a non-terpenoid constituent, comprised the major portion (41.6%) followed by β- caryophyllene (13.4%), spathulenol (14.6%), and myrcene (6.3%) (Manika et al. 2016). Perera et al. (2016) reported that the main constituents of *Lippia origanoides* EO were sesquiterpenes (45.3%) and cinnamate derivatives (41.7%), being the most effective EO tested by the authors (MIC = 0.078 mg/mL). Finally, the antifungal activity of *Baccharis parvidentata* against *C. neoformans* was evaluated (MIC = 1.25 mg/mL) being characterized by a high content of monoterpenes (51.9%) and sesquiterpenes (37.9%).

On the other hand, the combined effect of EOs and conventional antifungal drugs has been evaluated against *C. neoformans*. Tullio et al. (2019) reported a synergistic effect between *M. piperita* EO and itraconazole against *C. neoformans*. Moreover, Cardoso et al. (2017) combined *O. basilicum* ethanolic extract with amphotericic B, showing an increase in their anti-*Cryptococcis* activity. Capsule size, pigmentation, and ergosterol synthesis were also reduced. Similar results were reported in *C. neoformans* when *O. basilicum* EO and its main components, geraniol and linalool, were combined with fluconazole (Cardoso et al. 2016). Additionally, the synergistic effect of EOs and their main components in combination with chemical drugs against *C. neoformans* strains was evaluated by Scalas et al. (2018), who revealed the potential use of thyme and oregano EOs and carvacrol in combination with azoles for cryptococcosis treatment.

7 Aspergillosis

Aspergillosis represents a large and heterogeneous group of non-contagious opportunistic diseases caused by filamentous fungi of the genus *Aspergillus*. *Aspergillus* spp. are ubiquitous in the environment and grow as saprophytes, independently of an animal host. The human is constantly exposed to spores of *Aspergillus* which are effectively cleared by the immune system. However, in individuals with impaired immune functions, this exposure leads to the occurrence of invasive infections. The severity of the infection is the outcome of complex host–pathogen interactions, as well as the efficiency of the administered therapy. Among known *Aspergillus* spp., the majority of human infections are caused by *A. fumigatus*, followed by *A. flavus*, *A. terreus*, *A. nidulans,* and *A. niger*. *Aspergillus fumigatus* is the most important aerial fungal pathogen that causes invasive pulmonary infections in immunocompromised patients that often results in death (Latge and Chamilos 2019). The most commonly used drugs for the treatment of aspergillosis consist of antifungal agents that either target ergosterol, the main component of fungal membranes, or the synthesis of 1,3-glucan, the major component of the fungal cell wall (Latge and Chamilos 2019). The survival rates of immunocompromised patients with invasive aspergillosis have improved drastically with the use of azole antifungal drugs. However, the development of resistance mechanisms in *A. fumigatus* has increased

the mortality rates of patients with azole-resistant invasive aspergillosis from 50% to 100%, thus restricting the use of azole drugs (Verweij et al. 2016). The raising of resistant strains has resulted in the need for alternative therapeutic strategies, such as the use of new antifungal agents alone or in combinations with existing drugs. In this context, plant EOs and their active components have shown promising antifungal activities (Natu and Tatke 2019). Many EOs and EO pure components have been investigated for their antifungal activity against *A. fumigatus*. The MICs values of different EOs against *A. fumigatus* are shown in Table 10.5. Pure oxygenated compounds of phenolic nature report great antifungal activity, which is explained by the free hydroxyl group available to form hydrogen bonds with the active sites of different enzymes. Several EOs with high content of phenolic compounds were evaluated by different authors (Khan and Ahmad 2011b; Horváth et al. 2016; Ebani et al. 2017). The EOs from *T. vulgaris*, *O. vulgare*, and *Syzygium aromaticum* exerted a strong antifungal effect – thymol (46.3%), carvacrol (65.9%), and eugenol (74.3%) being their major constituents, respectively. The MIC of eugenol was identical to the MIC of *S. aromaticum* EO (0.32 mg/mL), indicating that this compound is responsible for the activity of the EO. This was not the case for thymol that showed a slightly higher MIC (0.19 mg/mL) than the MIC of *T. vulgaris* EO (0.14 mg/mL), which would indicate the presence of minor constituents in the EO that synergize the toxic effect of thymol (Khan and Ahmad 2011b). The EOs from different species belonging to the genus *Cymbopogon* showed high antifungal activity – their main constituents being aldehydes and alcohols monoterpenes, such as geranial and neral in *C. citratus* (74.0%), citronellal and geraniol in *C. nardus* (61.5%) and geraniol in *C. martini* (50.7%). The antifungal activity of EOs is usually attributable to their major components. However, minor constituents should not be underestimated because their presence may lead to additive, synergistic, or antagonistic effects. For example, the EO from *Litsea cubeba* showed a neral and geranial content very similar to *C. citratus*, but their MIC values were quite different (1.77 and 0.89 mg/mL, respectively), suggesting that other compounds might be influencing the bioactivity (Ebani et al. 2018). This is also the case of the EO from *Aloysia triphylla* which has a strong antifungal activity. Its chemical composition consists of 37.7% limonene, 24.0% sabinene, and 12.0% citronellal (Ebani et al. 2018). The high bioactivity is probably due to the combination of sabinene and citronellal, which proved effective against *A. fumigatus* (Aguiar et al. 2014; Roh and Shin 2014). On the contrary, its major component limonene is known for its weak inhibitory activity (Mahdavi Omran et al. 2011). This is in agreement with the high MIC values registered for different species of the genus *Citrus* that have limonene as their predominant component (Ebani et al. 2018). Cinnamaldehyde is the predominant component of *Cinnamomum zeylanicum* (56.4%) and *C. verum* EOs (79.1%) (Li et al. 2013; Ebani et al. 2018). This compound, as well as geraniol and citral, targets certain virulence factors of pathogenic fungi, such as elastasa and keratinasa activities (Khan and Ahmad 2011a), that destroy structural barriers during the infective process. Additionally, a fourfold increase of the MIC value of cinammaldehyde occurred in the presence of the osmotic protector sorbitol, suggesting that it might affect the fungal cell wall (Khan and Ahmad 2011a). Additionally, scanning and transmission

Table 10.5 Minimal inhibitory concentration of EOs against *Aspergillus fumigatus*

PLANT EO	Main components (%)	MIC	References
Cymbopogon nardus	Citronellal (36.2), Geraniol (25.3)	0.78 mg/mL	Horváth et al. (2016)
Cinnamomum zeylanicum	Cinnamaldehyde (74.0)	0.19 mg/mL	
Thymus vulgaris	Thymol (46.3), p-cCymene (22.1)	0.39 mg/mL	
	–	0.50 mg/mL	Puškárová et al. (2017)
	–	0.14 mg/mL	Khan and Ahmad (2011b)
	Thymol (52.6)	5.72 mg/mL	Ebani et al. (2017)
Syzygium aromaticum	–	0.25 mg/mL	Puškárová et al. (2017)
	Eugenol (88.6), β-Caryophyllene (8.6)	1.56 mg/mL	Horváth et al. (2016)
	Eugenol (74.3), β-Caryophyllene (7.0)	0.32 mg/mL	Khan and Ahmad (2011b)
Cymbopogon martini	Geraniol (50.7), Geraniol acetate (19.2)	0.14 mg/mL	
Cinnamomum verum	Cinnamaldehyde (79.1)	0.20 mg/mL	Moussaid et al. (2019), Li et al. (2013)
Cuminum cyminum	–	1.66 mg/mL	
	–	1.50 mg/mL	Khosravi et al. (2011)
Heracleum sphondylium	(Z)-β-Ocimene (28.9)	1.00 mg/mL (root)	Ušjak et al. (2017)
	Germacrene D (11.0) β-esquiphellandrene (10.6)	2.00 mg/mL (leaf)	
	Apiole (16.8), α-Acorenol (9.0)	0.50 mg/mL (flower)	
	Octyl acetate (67.1) n-Octanol (16.6),	1.50 mg/mL (fruit)	
Heracleum sibiricum	β-Pinene (26.2), Elemicina (25.6) Methyl eugenol (22.3)	0.30 mg/mL (root)	
	(Z)-Isoelemicin (16.6), Elemicina (14.9)	0.60 mg/mL (leaf)	
	Methyl eugenol (22.9), Elemicina (22.7), (Z)-Isoelemicin (18.5)	0.30 mg/mL (flower)	
	Octyl acetate (64.3) n-Octanol (21.1)	0.15 mg/mL (fruit)	
Heracleum montanum	(Z)-β-Ocimene (20.4)	0.50 mg/mL (root)	
	(E)-β-Farnesene (18.4), (E)-Caryophyllene (12.4)	2.00 mg/mL (leaf)	
	(E)-β-Farnesene (11.4), Sabinene (8.0)	0.50 mg/mL (flower)	
	Octyl acetate (57.5), n-Octanol (15.7)	3.00 mg/mL (fruit)	

(continued)

Table 10.5 (continued)

PLANT EO	Main components (%)	MIC	References
Abies holophylla	Bornyl acetate (19.4), Limonene (16.8), 3-carene (13.65), camphene (10.7), α-Pinene (10.4)	0.12 mg/mL	Jang et al. (2012)
Aloysia tryphilla	Limonene (36.7), Sabinene (24.0), Citronellal (12.0)	0.85 mg/mL	Ebani et al. (2018)
Citrus aurantium	Limonene (94.7)	8.50 mg/mL	
Citrus bergamia	Limonene (33.2), Linalyl acetate (31.7)	8.70 mg/mL	
Citrus limon	Limonene (65.7), γ-Terpinene (9.3)	4.25 mg/mL	
Citrus reticulata	Limonene (72.1), γ-Terpinene (19.2)	4.25 mg/mL	
Melaleuca alternifolia	4-Terpineol (30.2), γ-Terpinene (16.9)	1.78 mg/mL	
Eucalyptus globulus	1,8-Cineole (89.8)	4.58 mg/mL	
Cymbopogon citratus	Geranial (38.4), Neral (35.2)	0.89 mg/mL	
Litsea cubeba	Geranial (36.4), Neral (32.5)	1.77 mg/mL	
Pelargonium graveolens	Citronellol (44.5), Geraniol (13.7)	8.90 mg/mL	
Mentha piperita	Menthol (32.4), Menthone (26.6)	9.12 mg/mL	
Boswellia sacra	α-Thujene (54.2)	8.50 mg/mL	
Ocimum basilicum	Linalool (46.0), Eugenol (11.5)	2.29 mg/mL	Ebani et al. (2017)
Illicium verum	(E)-anethol (89.8)	0.59 mg/mL	
Rosmarinus officinalis	α-Pinene (37.9), 1,8-cineole (22.0)	0.29 mg/mL	
Salvia sclarea	Linalyl acetate (54.7), (Z)-8-Hydroxylinalool (15.8)	2.23 mg/mL	
Lavandula hybrida	Linalool (31.5), Linalyl acetate (26.8)	8.50 mg/mL	
Origanum vulgare	Carvacrol (65.9)	0.19 mg/mL	
	–	0.25 mg/mL	Puškárová et al. (2017)
Thuja plicata	–	0.75 mg/mL	
Lavandula viridis	1,8-Cineole (34.5), Camphor (13.4), α-Pinene (9.0)	2.50 μL/mL	Zuzarte et al. (2011)
Lavandula stoechas	Fenchone (37.0), Camphor (27.3)	1.25 μL/mL	Zuzarte et al. (2013)
Thymus herba barona	Carcacrol (54.0), Thymol (30.2)	0.16 μL/mL	
Oenanthe crocata	Trans-β-Ocimene (31.3), Sabinene (29.0)	1.25 μL/mL	Valente et al. (2013)
Seseli tortuosum	α-Pinene (24.9), β-Pinene (23.9), (Z)-β-Ocimene (13.3)	5.00 μL/mL	Gonçalves et al. (2012)
Seseli montanum	α-Pinene (36.0), β-Pinene (22.5), Limonene (8.8)	10.00 μL/mL	

electron micrographs of *A. fumigatus* treated with cinnamaldehyde showed a loss of integrity of the cell wall and membrane, expansion of endoplasmic reticulum, degeneration of mitochondria, autolysis, and degradation of cytoplasm content and an irregular distribution of polysaccharides. The pattern of ultrastructural alterations reported for this compound suggests multiple sites of action in fungi. The treatment of invasive pulmonary aspergillosis with cinnamaldehyde was highly effective in immunosuppressed mice (Deng et al. 2018). The survival rate of mices treated orally with cinnamaldehyde was significantly higher (80%) than the survival rate of mices treated with the positive control, voriconazole (60%). Additionally, the content of 1.3-glucan in lung tissues was significantly lower in mice treated with cinnamaldehyde compared to voriconazole, suggesting that cinnamaldehyde either interferes with the synthesis or destroys the integrity of the fungal cell wall (Deng et al. 2018).

As mentioned above, EOs can be obtained from different parts of the plants. The EOs from flowers, leaves, fruits, and roots from three different species belonging to the genus *Heracleum* were evaluated against *A. fumigatus* (Ušjak et al. 2017). Different major compounds were identified among species (and plant parts), being the EO from *Heracleum sibiricum* the one with lower MIC values. Particularly, the fruit of EOs was two- to four-fold more effective than the EOs from other plant parts of *H. sibiricum*, and 10- to 20-fold better than the fruit EO extracted from the other species (Ušjak et al. 2017).

Different interactions can occur among the components of an EO. Synergism represents a dynamic interplay of two or more compounds to enhance a bioactive effect. For example, the EO from *Abies holophylla* presents similar amounts of borneol, α-bisabolol, limonene, α-pinene, β-pinene, bornyl acetate, α-humulene, camphene, and caryophyllene (Jang et al. 2012). The MIC value of each pure compound was 0.25 mg/mL for borneol and α-bisabolol, and 0.5 mg/mL for the remaining compounds. The MIC value of the EO was significantly lower than 0.12 mg/mL, revealing a strong synergistic effect (Jang et al. 2012). Indeed, the mix of borneol and α-bisabolol reported a MIC of 0.12 mg/mL, lower than each single constituent, evidencing a synergistic effect. This pattern highlights the importance of hydroxyl groups-containing terpenes as antifungals. The sesquiterpene α-bisabolol inhibits *A. fumigatus* growth by affecting Δ24-sterol methyltransferase, a crucial enzyme in ergosterol biosynthesis pathway (Jahanshiri et al. 2017), and borneol causes severe damages to the cell wall (Lee et al. 2013). Not all the compounds of an EO are effective antifungal agents. In many cases, pure compounds show higher antifungal activity (lower MIC values) than the EO. For example, *Seseli tortuosum* and *Seseli montanum* EOs have modest antifungal activities (MIC = 5 μL/L and 10 μL/L respectively). However, antifungal assays using their major component, α-pinene reported a MIC value of 1.25 μL/L (Gonçalves et al. 2012).

The emergence of resistant strains has resulted in the need for novel antimycotic agents. Combinations of two or more antifungal agents have been performed to assess possible synergy and achieve a better therapeutic action. The degree of synergy between antimicrobial agents is often expressed in terms of the Fractional Inhibitory Concentration Index (FICI). According to Odds (2003), FICI $\leq$0.5

indicates synergy, FICI >0.5–4.0 indicates no interaction, and FICI >4.0 indicates an antagonistic effect. The EOs from *S. aromaticum* and *T. vulgaris* exhibit synergistic interactions with fluconazole against *A. fumigatus* (FICI = 0.250) (Khan and Ahmad 2011b). Regarding pure active compounds, eugenol shows a moderate synergism (FICI = 0.375) while thymol and cinnamaldehyde exhibit a stronger synergy with fluconazole (FICI = 0.187) against *A. fumigatus*. Fluconazole is one of the most efficient and safest antifungal drugs that affect the activity of the enzyme 14-α-demethylase, interrupting ergosterol biosynthesis (Khan and Ahmad 2011b). The synergistic interactions of EOs or their active compounds with fluconazole may be related to the simultaneous effects on different target sites by new agents and fluconazole, which is the desirable effect of combination therapies. Further experiments are needed to evaluate the therapeutic potential of these natural antifungal agents in combination with existing drugs against *A. fumigatus*.

8 Conclusion

The incidence of fungal infections has been continuously increasing over the last decades, with a high rate of death among patients with impaired immune systems. Furthermore, the rising of resistant strains has encouraged the development of new therapeutic strategies. Essential oils are composed of volatile organic compounds of different chemical nature mainly monoterpenes, sesquiterpenes, or phenylpropanoids, which proved to be effective for the treatment of invasive and superficial mycoses that affect human health. In addition, the synergistic activity of EOs and their pure components with existing antifungal drugs has been widely reported. The discovery of synergistic mechanisms to be exploited in combinational therapies will decrease the doses of synthetic drugs, thus reducing the development of resistance.

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