Chapter 19
Essential Oil Constituents: Biodiversity and Their Applicability for Cancer Therapy

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Abstract Essential oils are odoriferous substances traditionally used in the perfumery, food, and pharmaceutical industries. The most volatile fraction (constituting 90–95 % of total oil) comprises, in most cases, complex mixtures that may contain hundreds of compounds, which are composed mainly of terpenoids. Each of these constituents contributes for the biological effects of these essential oils. In this chapter, a total of 20 essential oil constituents, which presented positive results on cytotoxic drug screening, were selected; among them, ascaridole, α-bisabolol, (*E*)-caryophyllene, β-elemene, β-eudesmol, D-limonene, terpinen-4-ol, and thymol have been extensively studied with promissory results. Herein, we highlighted the recent advances in the knowledge of the chemical and anticancer properties of these compounds, establishing new goals for future research.

19.1 Introduction

Plant secondary metabolites have amazing structural diversity and biological activities, including anticancer proprieties [1, 2]. Among these, essential oils are odoriferous substances traditionally used in the perfumery, food, and pharmaceutical industries. Essential oils may be found in different plant parts generally in flowers (e.g. *Acacia* spp., *Dianthus caryophyllus*, *Jasminum* spp., *Lavandula* spp., *Rosa* spp., *Rosmarinus officinalis*, *Syzygium aromaticum*, etc.), fruits (*Citrus* spp., *Juniperus communis,* etc.), leaves (e.g. *Cymbopogon* spp., *Mentha* spp., *Ocimum* spp., etc.), leaves and stems (e.g. *Aloysia citriodora*, *Cinnamomum* spp., *Pelargonium* spp., *Pogostemon cablin*, etc.). Moreover, bark (e.g. *Betula*

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pubescens, Cinnamomum cassia), rhizomes (e.g. *Acorus calamus*, *Curcuma longa*, *Zingiber officinale*, etc.), roots (e.g. *Angelica archangelica*, *Vetiveria zizanioides*, etc.), seeds (e.g. *Coriandrum sativum*, *Foeniculum vulgare*, *Myristica* spp., etc.), and wood (e.g. *Aniba rosaeodora*, *Eremanthus erythropappus, Pinus sylvestris, Santalum album*, etc.) are also natural sources of commercially important essential oils [3, 4].

The most volatile fraction (constituting 90–95 % of total oil) comprises, in most cases, complex mixtures that may contain hundreds of compounds which are composed mainly of terpenoids (mono-, sesqui-, and even diterpenes). Besides, it may contain benzenoids, phenylpropanoids, aliphatic aldehydes, alcohols, and esters. Terpenoids (mono- and sesquitepenes) are the primary constituents of the essential oils of many types of herbs; many of them are commercially important, and are widely used as flavoring agents, perfumes, insecticides, antimicrobial agents, and raw material for important chemicals [5].

In this chapter, a total of 20 essential oil constituents, which presented positive results on cytotoxic drug screening, were selected (Fig. 19.1). In addition, we highlighted the recent advances in the knowledge of the chemical and anticancer properties of these compounds, establishing new goals for future research.

19.2 Chemical and Botanical Data

A rigid scheme for classifying secondary metabolites is not applicable due to their immense structural diversity; however, three main classes are often used: terpenoids and steroids; fatty-acid derivatives and polyketides; and alkaloids. Terpenoids constitute the largest and one of the most diverse classes of secondary metabolites and they are classified according to the number of containing five-carbon units coupled through biosynthetic pathways [6, 7]. In volatile fraction, we found terpenoids that are classified as hemiterpenes (C_5), monoterpenes (C_{10}), sesquiterpenes (C_{15}), and even some diterpenes (C_{20}).

Despite their diversity, all plant terpenoids derive from the common building units isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) that are synthesized via two parallel pathways known as mevalonate (MVA) and methylerythritol 4-phosphate (MEP) [6]. In general, MVA pathway leads to the synthesis of some sesquiterpenes and triterpenes (sterols) in the cytoplasm, while the MEP pathway is responsible for the synthesis of monoterpenes, diterpenes, tetraterpenes (carotenoids) and polyterpenes [7, 8].

In the most volatile fraction of essential oils, terpenoids are generally unsaturated compounds which easily decomposable by light, heat or oxygen to produce undesirable compounds. So, the steps of isolation, concentration and purification in methods for extraction of essential oil become critical. The most commonly used technique is the so-called traditional methods [9], i.e., those based on mechanical pressing (e.g. citrus oils) and by hydro- or steam-distillation. Steam distillation is the worldwide procedure for extraction of essential oils from plant

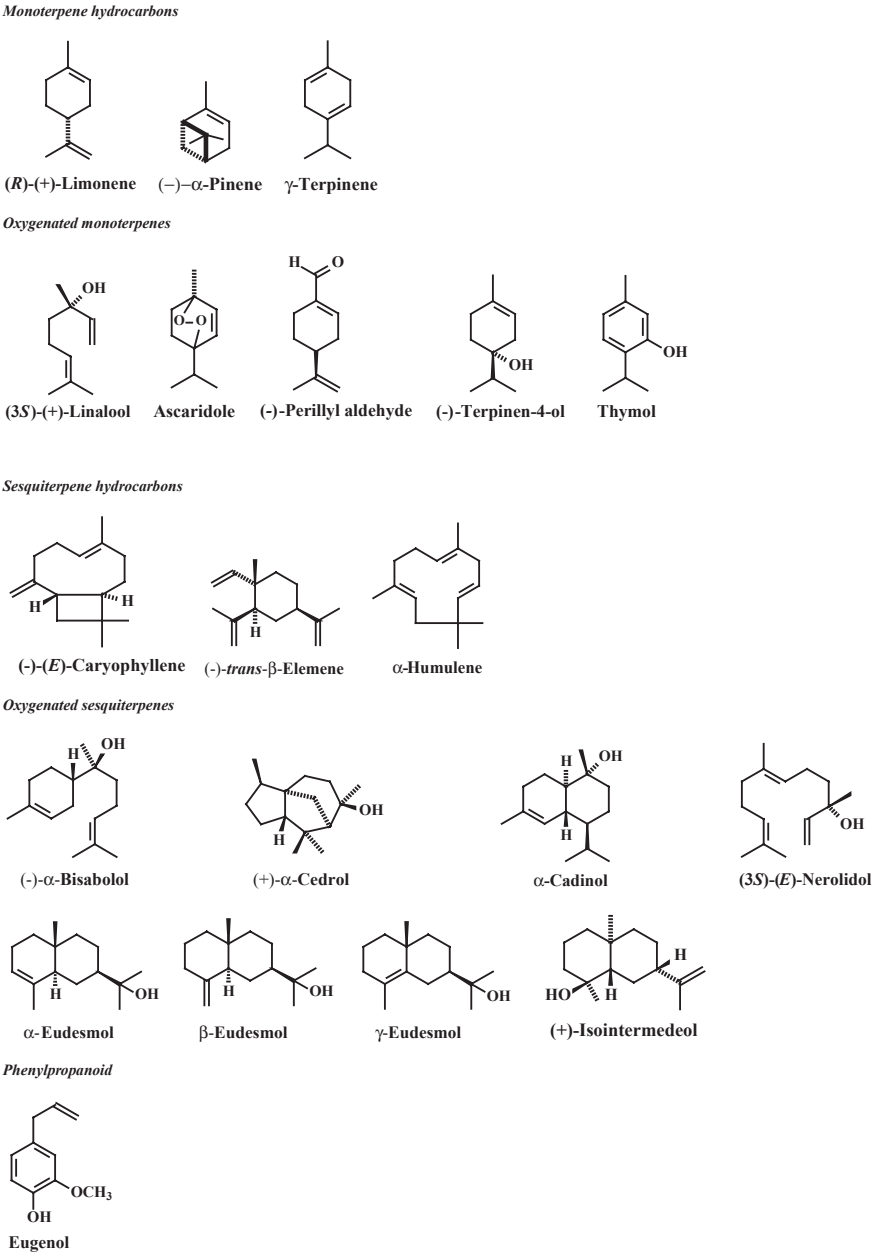

Fig. 19.1 Chemical structures of selected essential oil components

material, which is usually made by Clevenger-type apparatus in laboratory scale (hydro-distillation). The main drawback of this technique is related to the decomposition of labile compounds and the possibility of formation of non-natural

compounds (artifacts). Therefore, it is desirable to employ mild conditions to avoid oxidation, thermal deterioration or other chemical changes. Modern methods of sampling using small volume or no organic solvent have emerged in recent years. Techniques such as supercritical fluid extraction with CO_2 (SFE-CO_2), microwave assisted extraction (MAE) and pressure liquid extraction (PLE) have gained in interest as a green approach in volatile fraction extractions which can be used in small and large scale. These techniques have advantages and limitations, which have been recently reviewed [10–12].

Gas chromatograph (GC) coupled with a mass spectrometer (MS) has been the main analytical technique for the chemical characterization of essential oils [10] and together forms a powerful tool for high quality quantitative and qualitative analysis. However, GC/MS analysis of this complex mixture often is a time-consuming task due to limitations in techniques and instrumentation. Advances in this field have led to increased sensitivity, reproducibility and shorter times for analysis of volatile components. For example, fast GC often yields faster analysis times than conventional GC while maintaining resolution and allowing more samples to be analyzed per shift. Nevertheless, the use of fast GC for essential oils analysis is still exploring [13, 14].

For identification of essential oil constituents, computerized matching of acquired mass spectrum with those stored in the mass spectral libraries (NIST and Wiley) and with that of an authentic standard, comparison of retention indices [15] determined at least on two columns with distinct polarities together are currently the most widely used for structural identification [7]. Quadrupole mass spectra obtained by electron ionization (EI) at 70 eV has been preferred to the ion-trap-derived mass spectra due to resulting in fragmentation characteristics of each compound [16], especially for the identification of unknown compounds. Tandem mass spectrometry (MS–MS), Fourier transform infrared spectroscopy (FT-IR) and time-of-flight mass spectrometry (TOF–MS) are alternative techniques for detection employed to identify closely related isomers or overlapping compounds. The latter has been the detector of choice for GC × GC analysis [10, 17].

In addition, enantiomer separation and determination of enantiomeric ratio or enantiomeric excess (*e.e.*) remains with increasing interest mainly due to possible difference in enantiomer biological properties [18]. Two-dimensional (2D) chromatographic approach has been required for chiral recognition of components in complex samples. The most used techniques is heart-cutting multidimensional GC (MDGC) and comprehensive 2D GC (GC × GC). Advantages and limits of both techniques are well-known, although recently mass spectrometry as a second dimension in detection has gained further interest because of the role it can play in speeding up enantioselective GC analysis (es-GC) [18, 19]. Derivatized cyclodextrins (CDs) based columns are the most popular chiral stationary phase for es-GC [20] and has been successfully applied in the 2D GC analysis of chiral components from essential oils [21].

Recently there has been growing interest in terpenoids compounds due to the biological activity shown by some of them, especially as anticancer agents. In addition, terpenoid enantiomers are particularly useful chiral building blocks for

the chemical and biotechnological syntheses [22]. Thus, essential oils are a rich source of highly value compounds and their commercialization has increasing because they are also used widely in prevent and therapy several diseases [23].

Despite the importance of the issue, to our knowledge, little attention was paid on the investigation of chiral components of essential oils and their anticancer activity [24, 25].

It is widely known that many substances produced by living organisms are chiral and can occur alone (only one enantiomer) or can be present in varying amounts or even as racemic mixtures, exerting the same or distinct activities. For example, (4*S*)-(+)-carvone smell like caraway and (4*R*)-(−)-carvone has a distinct sweet spearmint odor with both enantiomers presenting different effects on the central nervous system [26]. Moreover, the isomers of the monoterpene alcohol (3*R*)-(−)- and (3*S*)-(+)-linalool have distinctive odors [27], but show the same antifungal activity [28].

The biosynthetic pathways of the essential oil components can produce one form only of an optically active chemical. Sometimes one form of an enantiomer may be produced in larger amounts, but often the relative abundances of both enantiomeric forms are very specific to the species and geographical origin of an essential oil [29].

Except for α-humulene, γ-terpinene, eugenol, and thymol exists chirality (stereogenic centers) in the other molecules mentioned in Fig. 19.1. Moreover, enantiomeric distribution of constituents from essential oils is still scarce, especially for sesquiterpenes. Nevertheless, some papers have been published mainly on the enantiomeric variation of monoterpenes such as (±)-limonene, (±)-linalool, (±)-α-pinene, and (±)-terpinen-4-ol [27, 28, 30, 31].

The enantiomers of the **linalool are found in variable** distribution from different plant species [21, 28]. In general, the optically active (3*S*)-(+)- and (3*R*)-(−)-linalool were isolated from lavender oil and coriander oil respectively, but both forms can be found in variable proportion in the wood and leaves essential oils of Brazilian rosewood (*Aniba rosaeodora* Ducke) [32]. It is noteworthy that the chiral stability may be influenced by the developmental stage of plant material, pH value and sampling techniques. In comparison, increasing amounts of the (3*S*)-(+) linalool was detected in oils produced by hydro-distillation of longer than 1 h duration [33]. In the same way, ascaridole, a monoterpene endoperoxide found as major component (up to 92 % of total oil) of essential oil from *Chenopodium ambrosioides* from various origins, is a heat-sensitive compound which rearranges to isoascaridole [34].

The enantiomeric composition of the monoterpene limonene is different for the various plant parts and changes during the development of the umbels as well as antimicrobial efficacy of the individual enantiomers and the racemic mixture showed variation [35]. For example, (4*R*)-(+)-limonene, the major component orange and other citrus peel oils, is one of the most investigated monoterpenes regarding the prevention of chemically induced tumors [36]. From a biosynthetic point of view, (4*S*)-(−)-limonene then serves as a precursor to other oxygenated monocyclic monoterpenes such as (−)-perillyl alcohol and (−)-perillaldehyde

which are minor components in many aromatic plants including *Perilla frutescens* (Lamiaceae) and also have been linked to anticancer activity [23, 24, 36].

The sesquiterpene α-(−)-bisabolol was first isolated from the blossoms of *Matricaria chamomilla* (Asteraceae) which may exist in three others possible stereoisomers: (+)-α-bisabolol and (+)- and (−)-*epi*-α-bisabolol. Besides, chamomile, another source of α-(−)-bisabolol is sage (*Salvia runcinata*) that contain up to 90 % and candeia (*Eremanthus erythropappus*) which may contain up to 85 % from the wood [37].

(−)-*trans*-β-elemene is a sesquiterpene that has attracted attention due to recent developments on their use in the antitumor therapy of many kinds of cancer. A comprehensive review that includes its natural occurrence, biogenesis, anticancer activity, and synthesis and chemical characterization was recently published [38].

Caryophyllene is probably the most widely distributed sesquiterpene in nature. The pure form of the (−)-(*E*)-caryophyllene is frequently present in the essential oil from *Humulus lupulus*, *Piper nigrum*, *Syzygium aromaticum* leaf, and *Copaifera officinalis*, etc. However, (+)-(*E*)-caryophyllene is rare and has been found in essential oils of liverworts [39].

Phenylpropanoid volatile compounds are found in essential oil composition of many plant species presenting significant biological activities. One such compound is eugenol which has been used by humans since antiquity for food preservation and flavoring and for medicinal purpose. Essential oil from clove (*Syzigium aromaticum*) is the main natural source of eugenol (up to 90 % of total oil) [40].

19.3 Anticancer Proprieties

As already mentioned, we selected a total of 20 compounds that presented positive results on cytotoxic drug screening; among them, ascaridole, α-bisabolol, (*E*)-caryophyllene, β-elemene, β-eudesmol, D-limonene, terpinen-4-ol, and thymol have been extensively studied with promissory results. Therefore, a special attention was given to these compounds. Moreover, the relevant compounds were summarized and included in a condensed form in Table 19.1.

Ascaridole exhibits cytotoxic activity against leukemia, melanoma, brain, and colon cancer cell lines, as well as, multiple drug resistance cancer cell lines [41, 42]. It also presented in vivo antitumor in a sarcoma murine model with no toxic side effects [42]. More recently, cell cycle and DNA damage analyses revealed a remarkable NER (nucleotide excision repair) specificity of ascaridol. Ascaridole decreased the G_1 phase in three cells lines, but it caused G_2/M phase arrest only in NER-deficient cells. It also induced an increase in the subG_1 peak which was considerably higher in NER-deficient cells than in proficient cells. Moreover, DNA damage induction was substantially higher in NER-deficient cells. In addition, ascaridole led to a dose-dependent increase in intracellular levels of reactive oxygen species at cytotoxic concentrations, but only NER-deficient cells showed a strongly induced amount of 8-oxodG sites [43].

Table 19.1 Summary of the anticancer proprieties of essential oil constituents

No.	Compounds	Anticancer proprieties	Range of IC_{50} values (μg/ml)	References
1	Ascaridole	In vitro and in vivo antitumor activities against several tumor cell lines G_2/M phase arrest and DNA-damage in NER-deficient cells Increase in intracellular levels of reactive oxygen species	6.3–18.4	[41–43]
2	α-Bisabolol	Selective cytotoxic effect against several tumor cell lines Apoptotic activity by liberation of cytochrome c and via Fas receptor	22.8–39.9	[44–46]
3	α-Cadinol	In vitro cytotoxic activity against several tumor cell lines	0.78–13.05	[82]
4	(*E*)-Caryophyllene	Selective cytotoxic effect against several tumor cell lines Induction of apoptosis by caspase-3 catalytic activity	20.1–21.8	[47–49]
5	α-Cedrol	In vitro cytotoxic activity against several tumor cell lines	41–44	[84]
6	β-Elemene	In vitro and in vivo antitumor activity against several tumor cells G_2/M phase arrest and apoptosis by reduction of Bcl-2 protein expression Enhances of taxanes- and cisplatin-induced cytotoxicity	45.3–56.3	[50–59]
7	α-Eudesmol	In vitro cytotoxic activity against several tumor cell lines	5.1–19.4	[64]
8	β-Eudesmol	In vitro and in vivo antitumor activity against several tumor cells Induces apoptosis by mitochondrial apoptotic pathway via JNK signaling Inhibits angiogenesis by blocking extracellular regulated protein kinases (ERK) MAPK signaling	20–25.1	[62–65]
9	γ-Eudesmol	In vitro cytotoxic activity against several tumor cell lines	0.01–20.6	[64, 85]

(continued)

Table 19.1 (continued)

No.	Compounds	Anticancer proprieties	Range of IC_{50} values (μg/ml)	References
10	Eugenol	In vitro and in vivo melanoma growth inhibition through inhibition of E2F1 transcriptional activity Apoptosis induction by translocation of phospho-ser 15-p53 into mitochondria	~0.08	[86, 87]
11	α-Humulene	In vitro cytotoxic activity against breast tumor cell line Induction of the decrease in cellular GSH content and increase in ROS production	~ 14.9	[87]
12	Isointermedeol	In vitro cytotoxic activity against tumor cell line Induction of apoptosis by both intrinsic and extrinsic pathways	~20	[88]
13	D-limonene	Inhibition of the growth of gastric cancer cell through apoptotic pathways Anti-angiogenic activity by down-regulation of VEGF	34.1–681.2	[66–71]
14	Linalool	In vitro cytotoxic activity against tumor cell line	~23	[83]
15	(*E*)-Nerolidol	In vitro cytotoxic activity against tumor cell lines	5.8–6.4	[89]
16	α-Pinene	In vitro cytotoxic activity against tumor cell line	~186.0	[90]
17	Perillaldehyde	In vitro cytotoxic activity against tumor cell lines	37.6–751.1	[91]
18	γ-Terpinene	In vitro cytotoxic activity against tumor cell line	~ 156.9	[90]
19	Terpinen-4-ol	Selective cytotoxic effect against several tumor cell lines Cell-cycle arrest and cell death through p53-dependent apoptosis pathways In vivo antitumor activity against xenograft tumor	54.84–189.7	[72–74]
20	Thymol	Induction of caspase-dependent and -independent apoptosis and necrosis Induces a $[Ca^{2+}]_i$ rise by inducing phospholipase C- and protein kinase C-dependent Ca^{2+} release from the endoplasmic reticulum and Ca^{2+} entry via non store-operated Ca^{2+} channels	~ 60.1	[79–81]

α-Bisabolol showed cytotoxic effect on several human and rat malignant cell lines. The action of α-bisabolol seems to be selective as its effect in normal mouse astroglial cells was not cytotoxic. It also exhibited apoptotic activity by induction of the liberation of cytochrome c and via Fas receptor [44, 45]. In addition, Gomes-Carneiro et al. [46] showed that α-bisabolol is non-mutagenic in the *Salmonella* microsomal test, and it can even neutralize the effect of various mutagenic substances. Moreover, α-bisabolol also showed an antigenotoxic effect against the hydrogen peroxide effect [45].

(*E*)-Caryophyllene has been reported to have cytotoxic activity over a wide range of tumor cell lines, but not against normal cells [47–49]. In addition, it caused an induction of apoptosis accompanied by DNA ladder and caspase-3 catalytic activity in tumor cell lines [49].

β-Elemene exhibits in vitro and in vivo antitumor activity on human and murine tumor cells. Many studies showed that the cell proliferation inhibited by β-elemene is correlated to G_2/M phase arrest and induction of apoptotic cell death by reduction of Bcl-2 protein expression. β-Elemene also enhances caspase-3 activity, and inhibits protein expression of eukaryotic initiation factors eIFs (4E, 4G), basic fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF) [50–57].

Furthermore, β-elemene markedly enhanced taxanes or cisplatin-induced cytotoxicity [57–59]. The combination treatments induced increased cytochrome c release from mitochondria, significant caspase-8 and -3 cleavage, and downregulation of Bcl-2 and Bcl-XL expression. The suppression of specific 'survival' gene expression appears to be the key action leading to the synergistic effect of combination treatments with β-elemene and taxanes [58, 59]. In vivo, the growth of laryngeal cancer cell-transplanted tumors in nude mice was inhibited by intraperitoneal injection of elemene. Compared with control groups, elemene significantly inhibited the protein expression of eIFs (4E and 4G), bFGF, and VEGF and decreased the microvessel density (MVD) [57]. Moreover, some clinical trials indicated that the possible side effects of β-elemene given intravenously include slight fever (usually lower than 38 °C), gastro-intestinal reactions, allergic reactions, local pain, and phlebitis. No bone marrow, liver, cardiac, or renal toxicities were found to be related to clinical treatment with β-elemene [50, 60, 61].

β-Eudesmol produced inhibitory effect on the growth of various tumor cells lines, but it had no effect on the proliferation of the rat aortic smooth muscle cells and astrocytes [62–64]. In addition, β-eudesmol induced apoptosis accompanied by cleavage of caspase-3, caspase-9, and poly (ADP-ribose) polymerase; downregulation of Bcl-2 expression; release of cytochrome c from mitochondria; and decrease in mitochondrial membrane potential (MMP). Activation of c-Jun N-terminal kinases (JNK) mitogen-activated protein kinases was observed in β-eudesmol-treated cells, and the inhibitor of JNK blocked the β-eudesmol-induced apoptosis, downregulation of Bcl-2, and the loss of MMP, suggesting that β-eudesmol induces apoptosis by mitochondrial apoptotic pathway, which is controlled through JNK signaling [63].

β-Eudesmol also inhibited angiogenesis by blocking extracellular regulated protein kinases (ERK) MAPK signaling [62]. Moreover, it inhibited the growth of mouse H22 and S180 tumor and the formation of new blood vessels in tumor tissues in vivo [65].

D-limonene is a known anticarcinogenic compound that it was proved to have antitumor activity [66–70]. In recent studies, D-limonene showed able to inhibit the growth of human gastric cancer cell in vitro through a mechanism of inducing the apoptosis of tumor cells [70].

In xenograft model, D-limonene alone or combined with 5-FU decreased drastically, the metastasis to liver, peritoneum and the occurrence of ascites were inhibited significantly compared with the control group. In addition, a notably decreased expression of MVD and VEGF in D-limonene and combined group were observed, suggesting the anti-angiogenic mechanism of D-limonene via down-regulation of VEGF [71].

Many studies have indicated that terpinen-4-ol exerts cytotoxic effects against several tumor cell lines without affecting normal cells. It is also able to induce cell-cycle arrest and cell death through apoptosis or necrosis pathways [72, 73]. In addition, Wu et al. [74] demonstrated that caspase-dependent mitochondrial dysfunction is the mechanism of terpinen-4-ol-induced apoptosis. Downregulation of Bcl-2, XIAP and survivin suggests that terpinen-4-ol increases the susceptibility of cancer cells to apoptosis induction. Notably, the ability of terpinen-4-ol to induce apoptosis in tumor cells was p53-dependent. Furthermore, the growth of s.c. xenograft tumors was remarkably inhibited by intratumoral injection of terpinen-4-ol, indicating that the agent also has potential for clinical anticancer activity.

The cytoprotective and antimutagenic effects of thymol has been extensively reported [75–78]. Anyway, some studies also have indicated the antitumor activity of thymol [79–81]. Thymol caused activation of caspase-9, -8 and -3 and concomitant PARP cleavage and it induced disruption of mitochondrial membrane potential, which is associated with caspase-dependent apoptosis. The disruption of mitochondrial membrane and activation of apoptosis appears to be dependent on reactive oxygen species. The translocation of AIF from mitochondria to cytosol and then to nucleus indicates thymols' ability to induce apoptosis through caspase independent pathway as well [79]. In addition, Hsu et al. [80] demonstrated that thymol induces a $[Ca^{2+}]$ concentration rise by inducing phospholipase C- and protein kinase C-dependent Ca^{2+} release from the endoplasmic reticulum and Ca^{2+} entry via non store-operated Ca^{2+} channels. Thymol induced cell death that may involve apoptosis.

19.4 Conclusions and Perspectives

A great amount of essential oil constituents with anticancer potential are found. The most of them presented cytotoxic activity only high range of IC_{50} values; therefore, they present weak clinical potential use. On the other hand, some of them have been extensively studied with promissory results. In short, 20

compounds were identified for their activities in the experimental models used for cytotoxic drug screening; among them, ascaridole, α-bisabolol, (*E*)-caryophyllene, β-elemene, β-eudesmol, D-limonene, terpinen-4-ol, and thymol have been shown promissory results. Anyway, further investigations are necessary to validate these compounds as novel clinically useful cancer chemotherapeutic agents.

References

1. Kinghorn AD, de Blanco EJC, Chai HB, Orjala J, Farnsworth NR, Soejarto DD, Oberlies NH, Wani MC, Kroll DJ, Pearce CJ, Swanson SM, Kramer RA, Rose WC, Fairchild CR, Vite GD, Emanuel S, Jarjoura D, Cope FO (2009) Discovery of anticancer agents of diverse natural origin. Pure Appl Chem 81:1051–1063
2. Adorjan B, Buchbauer G (2010) Biological properties of essential oils: an update review. Flavour Fragr J 25:407–426
3. Handa SS (2008) An overview of extraction techniques for medicinal and aromatic plants. In: Handa SS, Khanuja SPS, Longo G, Rakesh DD (eds) Extraction technologies for medicinal and aromatic plants. ICS-UNIDO, Trieste, pp 21–52
4. Bizzo HR, Hovell AMC, Rezende CM (2009) Brazilian essential oils: general view, developments and perspectives. Quim Nova 32:588–594
5. Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008) Biological effects of essential oils: a review. Food Chem Toxicol 46:446–475
6. Croteau R, Kutchan TM, Lewis NG (2000) Natural products (secondary metabolites). In: Buchanan B, Gruissem W, Jones R (eds) Biochemistry & molecular biology of plants. American Society of Plant Physiologists, Rockville, pp. 1250–1348
7. Tholl D, Boland W, Hansel A, Loreto F, Röse USR, Schnitzler JP (2006) Practical approaches to plant volatile analysis. Plant J 45:540–560
8. Takita MA, Berger IJ, Basílio-Palmieri AC, Borges KM, de Souza JM, Targon MLNP (2007) Terpene production in the peel of sweet orange fruits. Genet Mol Biol 30:841–847
9. Luque de Castro MD, Jiménez-Carmona MM, Fernández-Pérez V (1999) Towards more rational techniques for the isolation of valuable essential oils from plants. Trends Anal Chem 18:708–709
10. Marriot PJ, Shellie R, Cornwell C (2001) Gas chromatographic technologies for the analysis of essential oils. J Chromatogr A 936:1–22
11. Ormenõ E, Goldstein A, Niinemets Ü (2011) Extracting and trapping biogenic volatile organic compounds stored in plant species. Trends Analyt Chem 30:978–989
12. Sovilj MN, Nikolovski BG, Spasojevic MD (2011) Critical review of supercritical fluid extraction of selected spice plant materials. Maced J Chem Chem Eng 30:197–220
13. Matisová E, Dömötörová M (2003) Fast gas chromatography and its use in trace analysis. J Chromatogr A 1000:199–221
14. Rubiolo P, Liberto E, Sgorbini B, Russo R, Veuthey JL, Bicchi C (2008) Fast-GC-conventional quadrupole mass spectrometry in essential oil analysis. J Sep Sci 31:282–290
15. Van Den Dool H, Kratz PD (1963) A generalization of the retention index system including linear temperature programmed gas–liquid partition chromatography. J Chromatogr 11:463–471
16. Adams RP (2007) Identification of essential oil components by gas chromatography/mass spectrometry, 4th edn. Allured, Carol Stream
17. Baharum SN, Banawan H, Ghani MA, Mustapha WAW, Noor NM (2010) Analysis of the chemical composition of the essential oil of *Polygonium minus* Huds. using two-dimensional gas chromatography-time-of-flight mass spectrometry (GC-TOF-MS). Molecules 15:7006–7015

18. Bicchi C, Cagliero C, Rubiolo P (2011) New trends in the analysis of the volatile fraction of matrices of vegetal origin: a short overview. A review. Flavour Fragr J 26:321–325
19. Tranchida PQ, Sciarrone D, Dugo P, Modello L (2012) Heart-cutting multidimensional gas chromatography: a review of recent evolution, applications, and future prospects. Anal Chim Acta 716:66–75
20. Schurig V (2002) Chiral separations using gas chromatography. Trends Anal Chem 21:647–661
21. König WA, Hochmuth DH (2004) Enantioselective gas chromatography in flavor and fragrance analysis: strategies for the identification of known and unknown plant volatiles. J Chromatogr Sci 42:423–439
22. De Carvalho CCCR, da Fonseca MMR (2006) Biotransformation of terpenes. Biotechnol Adv 24:134–142
23. Edris AE (2007) Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review. Phytother Res 21:308–323
24. Elson CE, Yu SG (1994) The chemoprevention of cancer by mevalonate-derived constituents of fruits and vegetables. J Nutr 124:607–614
25. Bicas JL, Neri-Numa IA, Ruiz ALTG, De Carvalho JE, Pastore GM (2011) Evaluation of the antioxidant and antiproliferative potential of bioflavors. Food Chem Toxicol 49:1610–1615
26. Souza DP, Nóbrega FFF, Almeida RN (2007) Influence of the chirality of (*R*)-(−)- and (*S*)-(+)-carvone in the central nervous system: a comparative study. Chirality 19:264–268
27. Chanotiya CS, Yadav A (2009) Enantiomeric composition of (3R)-(−)- and (3S)-(+)-linalool in various essential oils of Indian origin by enantioselective capillary gas chromatography-flame ionization and mass spectrometry detection methods. Nat Prod Commun 4:563–566
28. Özek T, Tabanca N, Demirci F, Wedge DE, Baser KHC (2010) Enantiomeric distribution of some linalool containing essential oils and their biological activities. Rec Nat Prod 4:180–192
29. Dickschat JS (2011) Isoprenoids in three-dimensional space: the stereochemistry of terpene biosynthesis. Nat Prod Rep 28:1917–1936
30. Del Castillo MLR, Caja MM, Blanch GP, Herraiz M (2003) Chiral evaluation of aroma-active compounds in real complex samples. J Food Sci 68:770–774
31. Bonaccorsi I, Sciarrone D, Cotroneo A, Mondello L, Dugo P, Dugo G (2011) Enantiomeric distribution of key volatile components in *Citrus* essential oils. Braz J Pharmacogn 21:841–849
32. Zellner BD, Presti ML, Barata LES, Dugo P, Dugo G, Mondello L (2006) Evaluation of leaf-derived extracts as an environmentally sustainable source of essential oils by using gas chromatography-mass spectrometry and enantioselective gas chromatography-olfactometry. Anal Chem 78:883–890
33. Kreis P (1992) Chiral compounds of essential oils. Part XII. Authenticity control of rose oils using enantioselective multidimensional gas chromatography. Flavour Fragr J 7(4):199–203
34. Cavalli J-F, Tomi F, Bernardini A-F, Casanova J (2004) Combined analysis of the essential oil of *Chenopodium ambrosioides* by GC, GC-MS and ^{13}C-NMR spectroscopy: quantitative determination of ascaridole, a heat-sensitive compound. Phytochem Anal 15(5):275–279
35. Van Vuuren SF, Viljoen AM (2007) Antimicrobial activity of limonene enantiomers and 1,8-cineole alone and in combination. Flavour Fragr J 22:540–544
36. Miller JA, Thompson PA, Hakim IA, Chow HHS, Thomson CA (2011) D-Limonene: a bioactive food component from citrus and evidence for a potential role in breast cancer prevention and treatment. Oncol Rev 5:31–42
37. Kamatou GPP, Viljoen AM (2010) A review of the application and pharmacological properties of α-bisabolol and α-bisabolol-rich oils. J Am Oil Chem Soc 87:1–7
38. Adio AM (2009) (−)-*trans*-β-elemene and related compounds: occurrence, synthesis, and anticancer activity. Tetrahedron 65:5145–5159
39. Fricke C, Rieck A, Hardt IH, König WA, Muhle H (1995) Identification of (+)-β-caryophyllene in essential oils of liverworts by enantioselective gas chromatography. Phytochemistry 39:1119–1121

40. Kamatou GP, Vermaak I, Viljoen AM (2012) Eugenol-from the remote Maluku islands to the international market place: a review of a remarkable and versatile molecule. Molecules 17:6953–6981
41. Efferth T, Olbrich A, Sauerbrey A, Ross DD, Gebhart E, Neugebauer M (2002) Activity of ascaridol from the anthelmintic herb *Chenopodium anthelminticum* L. against sensitive and multidrug-resistant tumor cells. Anticancer Res 22:4221–4224
42. Bezerra DP, Marinho Filho JD, Alves AP, Pessoa C, Moraes MO, Pessoa OD, Torres MC, Silveira ER, Viana FA, Costa-Lotufo LV (2009) Antitumor activity of the essential oil from the leaves of *Croton regelianus* and its component ascaridole. Chem Biodivers 6:1224–1231
43. Abbasi R, Efferth T, Kuhmann C, Opatz T, Hao X, Popanda O, Schmezer P (2012) The endoperoxide ascaridol shows strong differential cytotoxicity in nucleotide excision repair-deficient cells. Toxicol Appl Pharmacol 259:302–310
44. Cavalieri E, Mariotto S, Fabrizi C, de Patri AC, Gottardo R, Leone S, Berra LV, Lauro GM, Ciampa AR, Suzuki H (2004) Alphabisabolol, a nontoxic natural compound, strongly induces apoptosis in glioma cells. Biochem Biophys Res Commun 315:589–594
45. Anter J, Romero-Jiménez M, Fernández-Bedmar Z, Villatoro-Pulido M, Analla M, Alonso-Moraga A, Muñoz-Serrano A (2011) Antigenotoxicity, cytotoxicity, and apoptosis induction by apigenin, bisabolol, and protocatechuic acid. J Med Food 14:276–283
46. Gomes-Carneiro MR, Dias DM, De-Oliveira AC, Paumgartten FJ (2005) Evaluation of mutagenic and antimutagenic activities of alpha-bisabolol in the Salmonella/microsome assay. Mutat Res 585:105–112
47. Kubo I, Chaudhuri SK, Kubo Y, Sanchez Y, Ogura T, Saito T, Ishikawa H, Haraguchi H (1996) Cytotoxic and antioxidative sesquiterpenoids from *Heterotheca inuloides*. Planta Med 62:427–430
48. Ashour ML, El-Readi M, Youns M, Mulyaningsih S, Sporer F, Efferth T, Wink M (2009) Chemical composition and biological activity of the essential oil obtained from *Bupleurum marginatum* (Apiaceae). J Pharm Pharmacol 61:1079–1087
49. Amiel E, Ofir R, Dudai N, Soloway E, Rabinsky T, Rachmilevitch S (2012) β-Caryophyllene, a compound isolated from the biblical balm of gilead (*Commiphora gileadensis*), is a selective apoptosis inducer for tumor cell lines. Evid Based Complement Alternat Med 2012:872394
50. Yang H, Wang X, Yu L (1996) The antitumor activity of elemene is associated with apoptosis. Zhonghua Zhong Liu Za Zhi 18:169–172
51. Zou L, Liu W, Yu L (2001) beta-elemene induces apoptosis of K562 leukemia cells. Zhonghua Zhong Liu Za Zhi 23:196–198
52. Zhou HY, Shen JK, Hou JS, Qiu YM, Luo QZ (2003) Experimental study on apoptosis induced by elemene in glioma cells. Ai Zheng 22:959–963
53. Xu YH, Dong B, Luo QZ, Zhou HY, Jia YC, Yang YF, Wang YZ (2005) Influence of elemene on the expression of Bcl-2 family genes in rat C6 glioma cells. Zhonghua Yi Xue Za Zhi 85:1700–1703
54. Wang G, Li X, Huang F, Zhao J, Ding H, Cunningham C, Coad JE, Flynn DC, Reed E, Li QQ (2005) Antitumor effect of beta-elemene in non-small-cell lung cancer cells is mediated via induction of cell cycle arrest and apoptotic cell death. Cell Mol Life Sci 62:881–893
55. Wang XS, Yang W, Tao SJ, Li K, Li M, Dong JH, Wang MW (2006) Effect of delta-elemene on Hela cell lines by apoptosis induction. Yakugaku Zasshi 126:979–990
56. Zhou HY, Hou JS, Wang Y (2006) Dose-and time-dependence of elemene in the induction of apoptosis in two glioma cell lines. Zhonghua Zhong Liu Za Zhi 28:270–271
57. Tao L, Zhou L, Zheng L, Yao M (2006) Elemene displays anti-cancer ability on laryngeal cancer cells *in vitro* and *in vivo*. Cancer Chemother Pharmacol 58:24–34
58. Li X, Wang G, Zhao J, Ding H, Cunningham C, Chen F, Flynn DC, Reed E, Li QQ (2005) Antiproliferative effect of betaelemene in chemoresistant ovarian carcinoma cells is mediated through arrest of the cell cycle at the G_2-M phase. Cell Mol Life Sci 62:894–904
59. Zhao J, Li QQ, Zou B, Wang G, Li X, Kim JE, Cuff CF, Huang L, Reed E, Gardner K (2007) *In vitro* combination characterization of the new anticancer plant drug beta-elemene with taxanes against human lung carcinoma. Int J Oncol 31:241–252

60. Wang J, Zhang H, Sun Y (1996) Phase III clinical trial of elemenum emulsion in the management of malignant pleural and peritoneal effusions. Zhonghua Zhong Liu Za Zhi 18:464–467
61. Tan P, Zhong W, Cai W (2000) Clinical study on treatment of 40 cases of malignant brain tumor by elemene emulsion injection. Zhongguo Zhong Xi Yi Jie He Za Zhi 20:645–648
62. Tsuneki H, Ma EL, Kobayashi S, Sekizaki N, Maekawa K, Sasaoka T, Wang MW, Kimura I (2005) Antiangiogenic activity of beta-eudesmol in vitro and in vivo. Eur J Pharmacol 512:105–115
63. Li Y, Li T, Miao C, Li J, Xiao W, Ma E (2012) β-Eudesmol induces JNK-dependent apoptosis through the mitochondrial pathway in HL60 cells. Phytother Res 14. doi:10.1002/ptr.4727
64. Britto ACS, Oliveira ACA, Henriques RM, Cardoso GMB, Bomfim DS, Carvalho AA, Moraes MO, Pessoa C, Pinheiro MLB, Costa EV, Bezerra DP (2012) *In vitro* and in vivo antitumor effects of the essential oil from the leaves of *Guatteria friesiana*. Planta Med 78:409–414
65. Ma EL, Li YC, Tsuneki H, Xiao JF, Xia MY, Wang MW, Kimura I (2008) beta-Eudesmol suppresses tumour growth through inhibition of tumour neovascularisation and tumour cell proliferation. J Asian Nat Prod Res 10:159–167
66. Uedo N, Tatsuta M, Iishi H, Baba M, Sakai N, Yano H, Otani T (1999) Inhibition by D-limonene of gastric carcinogenesis induced by N-methyl-N′-nitro-N-nitrosoguanidine in Wistar rats. Cancer Lett 137:131–136
67. Broitman SA, Wilkinson J 4th, Cerda S, Branch SK (1996) Effects of monoterpenes and mevinolin on murine colon tumor CT-26 in vitro and its hepatic "metastases" in vivo. Adv Exp Med Biol 401:111–130
68. Kaji I, Tatsuta M, Iishi H, Baba M, Inoue A, Kasugai H (2001) Inhibition by D-limonene of experimental hepatocarcinogenesis in Sprague-Dawley rats does not involve p21(ras) plasma membrane association. Int J Cancer 93:441–444
69. Stratton SP, Dorr RT, Alberts DS (2000) The state-of-the-art in chemoprevention of skin cancer. Eur J Cancer 36:1292–1297
70. Lu XG, Feng BA, Zhan LB, Yu ZH (2003) D-limonene induces apoptosis of gastric cancer cells. Zhonghua Zhongliu Zazhi 25:325–327
71. Lu XG, Zhan LB, Feng BA, Qu MY, Yu LH, Xie JH (2004) Inhibition of growth and metastasis of human gastric cancer implanted in nude mice by D-limonene. World J Gastroenterol 10:2140–2144
72. Greay SJ, Ireland DJ, Kissick HT, Levy A, Beilharz MW, Riley TV, Carson CF (2010) Induction of necrosis and cell cycle arrest in murine cancer cell lines by *Melaleuca alternifolia* (tea tree) oil and terpinen-4-ol. Cancer Chemother Pharmacol 65:877–888
73. Calcabrini A, Stringaro A, Toccacieli L, Meschini S, Marra M, Colone M, Salvatore G, Mondello F, Arancia G, Molinari A (2004) Terpinen-4-ol, the main component of *Melaleuca alternifolia* (tea tree) oil inhibits the in vitro growth of human melanoma cells. J Invest Dermatol 122:349–360
74. Wu CS, Chen YJ, Chen JJ, Shieh JJ, Huang CH, Lin PS, Chang GC, Chang JT, Lin CC (2012) Terpinen-4-ol induces apoptosis in human nonsmall cell lung cancer in vitro and in vivo. Evid Based Complement Alternat Med 2012:818261
75. Archana PR, Nageshwar Rao B, Ballal M, Satish Rao BS (2009) Thymol, a naturally occurring monocyclic dietary phenolic compound protects Chinese hamster lung fibroblasts from radiation-induced cytotoxicity. Mutat Res 680:70–77
76. Mezzoug N, Elhadri A, Dallouh A, Amkiss S, Skali NS, Abrini J, Zhiri A, Baudoux D, Diallo B, El Jaziri M, Idaomar M (2007) Investigation of the mutagenic and antimutagenic effects of *Origanum compactum* essential oil and some of its constituents. Mutat Res 629:100–110
77. Horvathova E, Turcaniova V, Slamenova D (2007) Comparative study of DNA damaging and DNA-protective effects of selected components of essential plant oils in human leukemic cells K562. Neoplasma 54:478–483
78. Mahmud H, Mauro D, Foller M, Lang F (2009) Inhibitory effect of thymol on suicidal erythrocyte death. Cell Physiol Biochem 24:407–414

79. Deb DD, Parimala G, Saravana Devi S, Chakraborty T (2011) Effect of thymol on peripheral blood mononuclear cell PBMC and acute promyelotic cancer cell line HL-60. Chem Biol Interact 193:97–106
80. Hsu SS, Lin KL, Chou CT, Chiang AJ, Liang WZ, Chang HT, Tsai JY, Liao WC, Huang FD, Huang JK, Chen IS, Liu SI, Kuo CC, Jan CR (2011) Effect of thymol on Ca^{2+} homeostasis and viability in human glioblastoma cells. Eur J Pharmacol 670:85–91
81. Chang HT, Hsu SS, Chou CT, Cheng JS, Wang JL, Lin KL, Fang YC, Chen WC, Chien JM, Lu T, Pan CC, Cheng HH, Huang JK, Kuo CC, Chai KL, Jan CR (2011) Effect of thymol on Ca^{2+} homeostasis and viability in MG63 human osteosarcoma cells. Pharmacology 88:201–212
82. He K, Zeng L, Shi G, Zhao GX, Kozlowski JF, McLaughlin JL (1997) Bioactive compounds from *Taiwania cryptomerioides*. J Nat Prod 60:38–40
83. Loizzo MR, Tundis R, Menichini F, Saab AM, Statti GA, Menichini F (2008) Antiproliferative effects of essential oils and their major constituents in human renal adenocarcinoma and amelanotic melanoma cells. Cell Prolif 41:1002–1012
84. Hsieh TJ, Chang FR, Chia YC, Chen CY, Chiu HF, Wu YC (2001) Cytotoxic constituents of the fruits of *Cananga odorata*. J Nat Prod 64:616–619
85. Ghosh R, Nadiminty N, Fitzpatrick JE, Alworth WL, Slaga TJ, Kumar AP (2005) Eugenol causes melanoma growth suppression through inhibition of E2F1 transcriptional activity. J Biol Chem 280:5812–5819
86. Park BS, Song YS, Yee SB, Lee BG, Seo SY, Park YC, Kim JM, Kim HM, Yoo YH (2005) Phospho-ser 15-p53 translocates into mitochondria and interacts with Bcl-2 and Bcl-xL in eugenol-induced apoptosis. Apoptosis 10:193–200
87. Legault J, Dahl W, Debiton E, Pichette A, Madelmont JC (2003) Antitumor activity of balsam fir oil: production of reactive oxygen species induced by alpha-humulene as possible mechanism of action. Planta Med 69:402–407
88. Kumar A, Malik F, Bhushan S, Sethi VK, Shahi AK, Kaur J, Taneja SC, Qazi GN, Singh J (2008) An essential oil and its major constituent isointermedeol induce apoptosis by increased expression of mitochondrial cytochrome c and apical death receptors in human leukaemia HL-60 cells. Chem Biol Interact 171:332–347
89. Sylvestre M, Pichette A, Lavoie S, Longtin A, Legault J (2007) Composition and cytotoxic activity of the leaf essential oil of *Comptonia peregrina* (L.) Coulter. Phytother Res 21:536–540
90. Döll-Boscardin PM, Sartoratto A, Sales Maia BH, Padilha de Paula J, Nakashima T, Farago PV, Kanunfre CC (2012) *In vitro* cytotoxic potential of essential oils of *Eucalyptus benthamii* and its related terpenes on tumor cell lines. Evid Based Complement Alternat Med 2012:342652
91. Sonboli A, Esmaeili MA, Gholipour A, Kanani MR (2010) Composition, cytotoxicity and antioxidant activity of the essential oil *of Dracocephalum surmandinum* from Iran. Nat Prod Commun 5:341–344

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