

# Essential Oils for Food Application: Natural Substances with Established Biological Activities

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**Abstract** Essential oils (EOs) are natural products obtained from aromatic plants. Steam distillation and hydrodistillation are the most commonly used methods for the extraction of EOs at laboratory scale. They have been widely studied due to their potential in the food industry. EO can be used in food in order to prolong the shelf-life, and additionally, they can reduce or replace synthetic additives. Their effectiveness can be confirmed in antimicrobial and antioxidant tests performed, in general, by diffusion test in agar and DPPH<sup>•</sup> assay, respectively. Volatile compounds are present in EOs, a role in their biological activities. In this line of thought, chromatography techniques can be applied to identify the main volatile compounds present in EOs. In general, EOs extend food stability during storage, inhibiting the growth of spoilage or pathogenic microorganisms and protecting against oxidation. It is important to evaluate the responsible compounds for the biological activities of EOs and determine their utilization limits, including their safety. Highly variable composition with source species, plant parts, and/or extraction methods appears to play

important roles in the variability of EO biological activities. This review provides a concise and critical insight in the use of EOs with emphasis in food applications.

**Keywords** Additives · Volatile compounds · DPPH<sup>•</sup> · Gas chromatography · Analytical methods

## Introduction

In recent years, consumers' concern over the quality and safety of food has significantly increased. The increasing demand for healthier, nutritious, and safer foodstuffs is reflected in the research on newer technologies for their preservation during production, transport, and storage. In addition, efforts have been made in order to prevent foodborne diseases that affect thousands of people around the world and are a major public health concern (Centers for Disease Control and Prevention 2015; Organização Mundial de Saúde 2015).

In this sense, new alternatives from natural sources have been studied such as the use of essential oils (EOs). EOs are secondary metabolites from aromatic plants and have been the focus of extensive research, not only due to their natural nature but also because they have demonstrated benefits in food and in human health. EOs have been used for centuries as perfume fragrances, in culinary as flavoring, and in folk medicine. Currently, they are mainly studied for their different biological properties such as antioxidant, antimicrobial, antitumor, analgesic, insecticidal, antidiabetic, and antiinflammatory (Ocaña-Fuentes et al. 2010; Yen et al. 2015; Brahmi et al. 2016; Periasamy et al. 2016).

In general, the EOs' major components are the main responsible for their biological properties, but it is known that the minor compounds can also contribute for these properties and, together, they can exhibit a synergetic activity (Burt

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2004). In this line of thought, the identification and quantification of the EOs' active compounds are very important. The most common analytical technique used for the determination of volatile active compounds of EOs is gas chromatography (GC) coupled with mass spectrometry (MS).

In food systems, EOs have demonstrated antimicrobial and antioxidant capacity, which allows increasing shelf-life of the product and ensure its quality (Oussalah et al. 2004, 2007; Zinoviadou et al. 2009). Additionally, EOs have been well regarded as an alternative to reduce or replace the use of synthetic additives which are associated with various human health adverse effects (Proestos et al. 2006).

Factors such as lipid oxidation and microbial growth are responsible for the loss of quality and reduced expiration date of the food product. The oxidation causes the degradation of the basic structure of lipids, which are responsible for the texture and the taste of foods rich in fat, among others (Sanches-Silva et al. 2004). Food contaminated by fungi and bacteria has social and economic implications, not only because they reduce the quality of food but also because they cause foodborne diseases (Centers for Disease Control and Prevention 2015).

For this reason, a large variety of EOs from different aromatic plants has been applied to food or incorporated in food packaging (Oussalah et al. 2007; Zinoviadou et al. 2009; Salgado et al. 2013). In this study, an extensive review of scientific publications, in which EOs perform antioxidant and antimicrobial function, was conducted. Different EOs were reviewed and discussed in what regards to their biological functions, main active compounds, and techniques used for the study of their composition. The main objective of this review was to access the current status and the recent advances regarding the use of EOs in food systems.

## Essential Oils

EOs are aromatic substances produced by plants generally belonging to angiospermic families that can be used by several industries for different purposes (Pavela 2015). Thus, EOs are natural products obtained from aromatic plant materials such as flowers, buds, seeds, leaves, stem, bark, and herbs (Dvaranauskaitė et al. 2009; Aidi Wannes et al. 2010; Lv et al. 2012; Hill et al. 2013).

For EO preparation, there are different methods that can be used which can enhance or reduce the above-mentioned properties. EOs can be extracted by hydrodistillation, steam distillation, solvent extraction, microwave extraction, and supercritical fluid extraction. Steam distillation and hydrodistillation are the most commonly used methods for the extraction of EOs at laboratory scale (Khajeh et al. 2005; Edris 2007; Bendahou et al. 2008; Dvaranauskaitė et al. 2009; Romano et al. 2009).

The uses of EOs as natural food additives have been the focus of countless investigation reports due to the possibility of these active mixtures to replace the synthetic food additives. As mentioned previously, synthetic additives have negative side effects to human health and the consumers are beginning to be aware of those facts and are starting to reject products with non-natural additives (Cacho et al. 2016). These oils have preservative properties related to the presence of compounds with antimicrobial and antioxidant capacity in their composition, which minimize or eliminate the presence of microorganisms and/or reduce the lipid oxidation phenomenon. Additionally, EOs are considered as generally recognized as safe (GRAS) by the Food And Drug Administration (FDA) (2016).

A large variety of EOs from different plants such as basil (*Ocimum basilicum* L.), oregano (*Origanum vulgare* L.), cinnamon (*Cinnamomum zeylanicum* Blume), and rosemary (*Rosmarinus officinalis* L.) has been applied to food in order to extend food shelf-life (Zinoviadou et al. 2009; Viteri Jumbo et al. 2014; Gaio et al. 2015a; Ribeiro-Santos et al. 2015).

Several foods showed positive protection when using EO as additives such as meat, fish, and vegetables (Table 1).

## Active Compounds

The composition and quality of EOs are naturally affected by the plant characteristics, such as development stage, variety, geographical origin, part of the plant used, age, season, and condition of the plant when harvested. But they are also affected by the extraction method (namely the solvent used) and analysis conditions (Khajeh et al. 2005; Hussain et al. 2008; Negi 2012; Riahi et al. 2013). For instance, Elzaawely et al. (2007) revealed that the main components of the EO of *Alpinia zerumbet* obtained from flowers are different from the EO obtained from seeds.

The technique used to identify and quantify the compounds of the numerous EOs is, in general, GC coupled with MS detector (Khajeh et al. 2005; Riahi et al. 2013). These oils contain a mixture of compounds which includes terpenes and aroma compounds (phenols, aldehydes, alcohols, methoxy derivatives, and methylene dioxy compounds), being the oxygenated compounds responsible for the odor. This mixture of compounds provides the biological properties of EOs, but they are extremely dependent on external factors affecting the preparation procedure such as light, heat, and oxygen (Nakatsu et al. 2000; Handa et al. 2008; Diaz et al. 2012; Hossain et al. 2012; Tisserand and Young 2014; Pavela 2015). Major components can constitute up to 85% of an EO and, usually, define the biological properties of EOs. However, the other 15% are composed by minor components that, although are present in small levels, have a significant role in the biological activities acting in synergism with the

**Table 1** Essential oils applied to food

Food group/ food items	Essential oils	Biological properties	References
Fruits			
“Formosa” plum	Oregano ( <i>Origanum vulgare</i> ), bergamot ( <i>Citrus bergamia</i> )	Antimicrobial	(Choi et al. 2016)
Papaya	Oregano ( <i>O. vulgare</i> ), cinnamon ( <i>Cinnamomum zeylanicum</i> ), lemongrass ( <i>Cymbopogon flexuosus</i> )	Antimicrobial	(Espitia et al. 2012)
Strawberries	Clove ( <i>Syzygium aromaticum</i> ), mustard ( <i>Brassica nigra</i> ), lemon ( <i>Citrus limon</i> L.)	Antifungal	(Aguilar-González et al. 2015; Perdones et al. 2016)
Vegetable			
Broccoli	Asian spice; Italian spice	Antimicrobial	(Takala et al. 2013)
Fresh cabbage	Thyme ( <i>Thymus vulgaris</i> ), Oregano ( <i>O. vulgare</i> ), Lemongrass ( <i>Cymbopogon flexuosus</i> )	Antibacterial	(Hyun et al. 2015)
Minimally processed	Oregano ( <i>O. vulgare</i> ), Rosemary ( <i>Rosmarinus officinalis</i> )	Antimicrobial	(Azeredo et al. 2011)
Swiss chard leaves	Eucalyptus ( <i>Eucalyptus globulus</i> ), tea tree ( <i>Melaleuca alternifolia</i> ), clove ( <i>Syzygium aromaticum</i> )	Antimicrobial, Antioxidant	(Ponce et al. 2003, 2004)
Seafood			
Mediterranean octopus	Oregano ( <i>Origanum vulgare</i> )	Antimicrobial	(Atrea et al. 2009)
Fish	Clove ( <i>Syzygium aromaticum</i> )	Antimicrobial; Antioxidant	(Gómez-Estaca et al. 2010; Salgado et al. 2013)
Rainbow trout fillets	<i>Zataria multiflora</i> Boiss.	Antimicrobial; Antioxidant	(Raeisi et al. 2015)
Sardine	<i>Mentha suaveolens</i>	Antimicrobial	(Petretto et al. 2014)
Swordfish fillets	Thyme ( <i>T. vulgaris</i> )	Antimicrobial; Antioxidant	(Kykkidou et al. 2009)
Meat and Chicken			
Beef patties	Thyme ( <i>T. vulgaris</i> ), oregano ( <i>O. vulgare</i> )	Antimicrobial	(Emiroğlu et al. 2010)
Beef muscle	Oregano ( <i>O. vulgare</i> ); pimento	Antimicrobial	(Oussalah et al. 2004)
Bologna and ham	Cinnamon ( <i>Cinnamomum cassia</i> ); winter savory ( <i>Satureja montana</i> )	Antimicrobial	(Oussalah et al. 2007)
Chicken	Oregano ( <i>O. vulgare</i> )	Antimicrobial	(Fernández-Pan et al. 2014)
Meat	Oregano ( <i>O. vulgare</i> )	Antimicrobial	(Skandamis and Nychas 2002)
Sausage	Basil ( <i>Ocimum basilicum</i> )	Antibacterial	(Gaio et al. 2015b)
Others foods			
Cheese	Oregano ( <i>O. vulgare</i> )	Antimicrobial	(Otero et al. 2014)
Rice	<i>Laurus nobilis</i> ; <i>Syzygium aromaticum</i>	Antifungal	(Pilar Santamarina et al. 2016)

major constituents (Burt 2004; Pavela 2015). Table 2 compiles some EOs and their major compounds.

Terpenes are organic compounds present in large quantities in all kinds of plants, formed by isoprene (C<sub>5</sub>H<sub>8</sub>) units (Calogirou et al. 1999). Monoterpenes, such as linalool, *p*-cymene, and limonene, are C-10 abundant natural compounds that represent approximately 90% of EOs and are associated with their biological activity (Olagnier et al. 2007; Pavela

2015). Sesquiterpenes (C-15 terpenes), such as  $\alpha$ -cadinene,  $\beta$ -caryophyllene, and  $\beta$ -elemene, are also present in EOs, although in lower quantities (Pavela 2015).

### Biological Properties of Essential Oils

Since ancient times, EOs are used as fragrances and flavoring agents in the perfume and food industries (Nakatsu et al. 2000;

**Table 2** Some EOs and their major compounds

Essential oil	Main components	Ref. (s)
Basil ( <i>Ocimum basilicum</i> )	Methyl chavicol, linalool	(Bozin et al. 2006)
Black cumin ( <i>Nigella sativa</i> )	<i>p</i> -Cymene, thymoquinone, tricyclene, $\alpha$ -thujene	(Viuda-Martos et al. 2011)
Cinnamon	Fenchene, cinnamaldehyde, pinene	(Cooke et al. 2000)
Citronella	Geraniol, citronellal, citronelol	(Cooke et al. 2000)
Clove	Eugenol	(Velluti et al. 2003)
Coriander	Terpinene, <i>p</i> -cymene, pinene	(Cooke et al. 2000)
Eucalyptus	Cineole, pinene, limonene	(Cooke et al. 2000)
Fennel ( <i>Foeniculum vulgare</i> )	<i>Trans</i> -anethole, methyl chavicol	(Viuda-Martos et al. 2011)
Ginger	Terpenio, neral, geraniol	(Cooke et al. 2000)
Lavender ( <i>Lavandula officinalis</i> )	Linalool, linalyl acetate, camphor	(Viuda-Martos et al. 2011)
Lemongrass	Geraniol, neral	(Velluti et al. 2003)
Lime	Limonene, terpeniols	(Cooke et al. 2000)
Orange	Limonene, terpeniols	(Cooke et al. 2000)
Oregano ( <i>O. vulgare</i> )	Carvacrol, thymol, <i>p</i> -cimene	(Bozin et al. 2006; Velluti et al. 2003)
Palmarose	Geraniol	(Velluti et al. 2003)
Parsley ( <i>Petroselinum crispum</i> )	Caryophyllene oxide apiole, $\alpha$ -pinene, $\beta$ -pinene,	(Viuda-Martos et al. 2011)
Pennyroyal ( <i>Mentha pulegium</i> )	Menthone, pulegone	(Teixeira et al. 2012)
Thyme ( <i>T. vulgaris</i> )	Thymol, <i>p</i> -cymene, $\gamma$ -terpinene	(Bozin et al. 2006; Viuda-Martos et al. 2011)

Tung et al. 2008; Unlu et al. 2010; Viteri Jumbo et al. 2014; Ali et al. 2015). In the cosmetic industry, EOs are used for skin and hair care and perfumes due to their biological properties that can protect our bodies against exogenous or endogenous harmful agents (Aburjai and Natsheh 2003). As to their pharmaceutical and medicinal uses, they are present in traditional Indian (Ayurveda) and Chinese (Zhong Yo) medicines (Nakatsu et al. 2000). The biological properties of EOs have drawn attention for their anticarcinogenic potential and the possible suppression of tumors including glioma, colon and gastric cancer, liver tumor, pulmonary tumor, breast cancer, and leukemia. EOs are also known for their potential activity against cardiovascular diseases (like atherosclerosis and thrombosis), bacteria and virus, oxidation, and diabetes, and they are also used for aromatherapy and massage (Edris 2007). They are also well-known for their preservative and antioxidant properties, offering protection against various chronic diseases and they can be a natural alternative to conventional therapy (Table 3).

There is a growing interest in the study of natural products for the discovery of active compounds not only with antimicrobial activity but also with antioxidant capacity to extend the storage stability of food, by inhibiting the growth of microorganisms and protecting food from oxidation. Several authors have tested EOs as an alternative to the synthetic additives used in foods (Khajeh et al. 2005; Hossain et al. 2008, 2012; Djabou et al. 2013).

The variability of EOs (related with their chemical composition) influence, mainly, their distinctive physico-chemical properties. The biological effect of EOs is dependent on the concentration used (in general, high concentration increases the biological effect), the microorganisms tested (fungal are more sensitive than bacteria) (Singh et al. 2007), tests performed, and on the main constituents of the oil. However, minor components may be critical to the activity (Burt 2004).

The possibility of synergism between a combination of EOs and among their constituents increases their spectrum of action enhancing the EO effectiveness (Goñi et al. 2009; Azeredo et al. 2011; Souza et al. 2013). These synergism effects, between major and minor compounds present in the EOs, should be taken into consideration when assessing their biological activity (Bouaziz et al. 2009). An antagonistic effect may also be observed when the combined effect is lower than individual effect (Goñi et al. 2009). An additional effect can be generated when the combined effect of the EO compounds is the same as the sum of the individual effects. Synergism occurs when the combined effect of the substances is higher than the sum of their individual effects. These effects may depend on the concentration of individual components of the EOs and on the concentration of each EO in the mixture (Goñi et al. 2009). A synergism advantage is that the individual concentration required to achieve the same effect can be significantly lower than the combination of two or more EOs. It may result in the reduction of undesirable sensory impact

**Table 3** Several biological properties of different essential oils

Scientific name	Common name	Biological activity of the corresponding essential oil	Ref.
<i>Allium cepa</i> L.	Onion	Antimicrobial	(Ye et al. 2013)
<i>Aquilariacrassna</i> Pierre ex Lecomte	Agarwood	Antipancreatic cancer	(Dahham et al. 2016)
<i>Cinnamomum zeylanicum</i> Blume	Cinnamon	Antimicrobial	(Unlu et al. 2010)
<i>Cuminumcyminum</i> L.	Cumin	Hepatoprotective	(Mostafa et al. 2015)
<i>Eucalyptus globules</i> Labill.	Tasmanian blue gum	Antioxidant	(Luis et al. 2016)
<i>Foeniculum vulgare</i> Mill	Fennel	Insecticidal	(Pavela et al. 2016)
<i>Illicium verum</i> Hook. f.	Star anise	Colon cancer	(Asif et al. 2016)
<i>Lavandula angustifolia</i> Mill.	Lavender	Analgesic	(Ghods et al. 2015)
<i>Melissa officinalis</i> L.	Lemon balm	Antidiabetic	(Yen et al. 2015)
<i>Mentha pulegium</i> L.	Pennyroyal	Insecticidal	(Brahmi et al. 2016)
<i>Mentha rotundifolia</i> L.	Apple mint	Insecticidal	
<i>Nigella sativa</i> L.	Black cumin	Breast cancer	(Periasamy et al. 2016)
<i>Ocimum gratissimum</i> L.	Basil	Antitypanosomal and antiplasmodial	(Kpadonou Kpoviessi et al. 2014)
<i>Origanum vulgare</i> L.	Oregano	Antiinflammatory	(Ocaña-Fuentes et al. 2010)
<i>Rosmarinus officinalis</i> L.	Rosemary	Antioxidant, antimicrobial, ant-cancer, antiinflammatory, antidiabetic	(Ojeda-Sana et al. 2013; Ribeiro-Santos et al. 2015)
<i>Salvia lavandulifolia</i> Vahl.	Spanish sage	Treatment of neurodegenerative diseases	(Porres-Martínez et al. 2013)
<i>Saturejahortensis</i> L.	Savory	Antifungal	(Razzaghi-Abyaneh et al. 2008)
<i>Skimmialaureola</i> Franch.	Nazar Panra	Antinociceptive and antipyretic	(Muhammad et al. 2013)

due to the strong aroma of some oils (Stojkovic et al. 2013). Therefore, synergism and antagonistic relationships among the EOs components might affect positively and negatively, respectively, their biological activity (Adrar et al. 2016; Ud-Daula et al. 2016).

Stojković et al. (2013) studied and proved the antimicrobial synergism of a combination of thyme and oregano EOs. Azeredo et al. (2011) showed also the synergism of *O. vulgare* and *R. officinalis* EOs against bacteria: *Listeria monocytogenes*, *Yersinia enterocolitica*, and *Aeromonas hydrophilla* and additive effect of combination of these EOs against *Pseudomonas fluorescens*. Goñi et al. (2009), reported that combination of cinnamon and clove EOs showed an antagonism effect on the inhibition of *Escherichia coli*. However, at higher concentrations, these individual EOs and their combinations presented the same activity against the growth of *E. coli*. Thus, a synergistic, antagonistic or additive effect could be concentration-dependent (Goñi et al. 2009).

Adrar et al. (2016) showed a synergistic antioxidant interaction between  $\alpha$ -tocopherol and *Thymus numidicus* EO (Adrar et al. 2016). Pei et al. (2009) reported synergistic effects of the combination of components of several EOs and their antimicrobial interactions against *E. coli*. Synergistic effects were found between cinnamaldehyde/eugenol, thymol/

eugenol, carvacrol/eugenol, and thymol/carvacrol (Pei et al. 2009).

Thus, identification and quantification of the EO compounds followed by antioxidant and antimicrobial capacity assays of the EO and their individual compounds are important to evaluate the constituents that contribute the most for their antioxidant and antimicrobial properties and to evaluate possible synergism. In addition, a chemical profile can be reproduced in laboratory based on this knowledge.

### Essential Oils as Antimicrobial Agents

EOs have shown promising antimicrobial action against a wide range of microorganisms. Table 4 compiles the EOs tested against several microorganisms and their minimal inhibitory concentrations (MIC). The MIC can be defined as the lowest concentration of a sample at which the tested microorganisms do not demonstrate any visible growth (Bozin et al. 2006; Naik et al. 2010; Ye et al. 2013).

EOs components have been applied due to their capacity in generating pleasant odors and for providing an effective action against several food pathogens thereby reducing foodborne diseases (Bouaziz et al. 2009).

**Table 4** MIC of essential oils tested in vitro against several microorganisms

Common name and scientific name (plant species)	Part of the plant used	Microorganism	MIC	Units	Ref.	
Basil ( <i>Ocimum basilicum</i> )	Leaves, stems, and flowers	<i>E. faecalis</i>	0.75	mg ml <sup>-1</sup>	(Gaio et al. 2015b)	
		<i>Micrococcus luteus</i>	0.50			
		<i>Sarcina</i> sp.	0.75			
		<i>S. aureus</i>	1.0			
		<i>S. epidermidis</i>	0.75			
		<i>Streptococcus mutans</i>	0.75			
		<i>Acinetobacter</i> sp.	1.0			
		<i>Aeromonas</i> sp.	1.0			
		<i>Citrobacter freundii</i>	1.0			
		<i>E. coli</i>	0.75			
		<i>K. pneumoniae</i>	1.0			
		<i>Proteus mirabilis</i>	0.75			
		<i>Proteus vulgaris</i>	0.50			
	<i>Salmonella choleraesuis</i>	0.25				
	<i>Serratia marcescens</i>	0.75				
	<i>Shigella flexneri</i>	0.25				
	<i>Yersinia enterocolitica</i>	0.73				
	Aerial parts	<i>C. albicans</i>	30	μL ml <sup>-1</sup>	(Bozin et al. 2006)	
		<i>Trichophyton mentagrophytes</i>	8.3			
		<i>Trichophyton tonsurans</i>	8.0			
		<i>Trichophyton rubrum</i>	8.3			
		<i>Epidermophyton floccosum</i>	15			
		<i>Microsporum canis</i>	15.2			
<i>A. niger</i>		2.2–4.5	mg ml <sup>-1</sup>			(Hussain et al. 2008)
<i>B. subtilis</i>		0.8–1.4				
<i>E. coli</i>		1.6–2.6				
<i>S. aureus</i>		0.9–1.5				
<i>Pasteurella multocida</i>	1.7–2.3					
<i>Mucor mucedo</i>	3.8–5.1					
<i>F. solani</i>	2.7–4.9					
<i>Botryodiplodia theobromae</i>	2.3–4.6					
<i>Rhizoctonia solani</i>	2.9–5.0					
Bergamot ( <i>Citrus bergamia</i> )	Peel	<i>S. aureus</i>		0.125	μg ml <sup>-1</sup>	
		<i>B. subtilis</i>	0.125			
		<i>E. coli</i>	5.0			
		<i>S. cerevisiae</i>	2.5			
Black Cumin ( <i>Nigella sativa</i> )	Seeds	<i>Streptococcus</i> spp.	2.13–8.50	mg ml <sup>-1</sup>	(Jrah Harzallah et al. 2011)	
		<i>E. faecalis</i>	≥8.5			
		<i>Gemella haemolysins</i>	2.13–4.25			
		<i>Gemella morbillorum</i>	2.13			
“Casca de anta” ( <i>Drimys Angustifolia</i> )	Branch	<i>B. cereus</i>	125	μg ml <sup>-1</sup>	(Santos et al. 2013)	
		<i>S. aureus</i>	500			
		<i>Acinetobacter baumannii</i>	500			
		<i>E. coli</i>	2000			
		<i>Pseudomonas aeruginosa</i>	1000			
Cinnamon <sup>a</sup> ( <i>Cinnamomum cassia</i> )	NR	<i>Escherichia coli</i>	300	μg ml <sup>-1</sup>	(Ooi et al. 2006)	
		<i>Enterobacter arugenus</i>	600			
		<i>Vibrio cholerae</i>	150			
		<i>Vibrio</i>	75			

**Table 4** (continued)

Common name and scientific name (plant species)	Part of the plant used	Microorganism	MIC	Units	Ref.
Clove ( <i>Eugenia aryophyllus</i> )	NR	<i>Salmonella</i>	300	mg ml <sup>-1</sup>	(Prabuseenivasan et al. 2006)
		<i>Proteus vulgaris</i>	250		
		<i>Pseudomonas</i>	300		
		<i>Staphylococcus aureus</i>	600		
		<i>Candida</i> sp.	113–450		
		<i>Fusarium solani</i>	150		
		<i>Aspergillus</i> sp.	88–125		
		<i>Microsporium gypseum</i>	28.0		
		<i>Tricophyton rubrum</i>	25.0		
		<i>Tricophyton mentagrophytes</i>	37.5		
		<i>Etilingera fimbriobracteata</i>	Rhizomes		
<i>B. subtilis</i>	>3.2				
<i>K. pneumoniae</i>	>6.4				
<i>P. vulgaris</i>	>3.2				
<i>P. aeruginosa</i>	>1.6				
Garlic ( <i>Allium sativum</i> )	NR	<i>E. coli</i>	>1.6	mg ml <sup>-1</sup>	(Ross et al. 2001)
		<i>E. coli</i>	156		
		<i>B. subtilis</i>	19.5		
		<i>Bacillus spizizenii</i>	19.5		
		<i>S. aureus</i>	39		
		<i>C. albicans</i>	39		
		<i>S. cerevisiae</i>	2.4		
		<i>E. coli</i>	0.34–5.5		
		<i>Salmonella entérica</i>	2.75–5.5		
		<i>L. monocytogenes</i>	0.02		
		<i>B. cereus</i>	0.08		
		<i>Shigella</i> sp.	1.37–2.75		
		<i>Vibrio</i> sp.	0.02–2.75		
		<i>Yersinia enterocolitica</i>	0.17–0.34		
		<i>Campylobacter</i> sp.	0.16–0.32		
		<i>Bacteroides fragilis</i>	0.02–0.04		
		<i>Bacillus subtilis</i>	0.17		
<i>Enterobacter aerogenes</i>	0.68				
<i>Enterococcus faecalis</i>	0.34				
<i>Klebsiella aerogenes</i>	0.04				
<i>Proteus vulgaris</i>	1.37				
<i>Lactobacillus acidophilus</i>	0.17–2.75				
<i>Streptococcus faecalis</i>	0.02–0.34				
Geranium ( <i>Pelargonium graveolens</i> )	NR	<i>S. aureus</i>	>12.8	mg ml <sup>-1</sup>	(Prabuseenivasan et al. 2006)
		<i>B. subtilis</i>	>6.4		
		<i>K. pneumoniae</i>	>12.8		
		<i>P. vulgaris</i>	>12.8		
		<i>P. aeruginosa</i>	>12.8		
Lemon ( <i>Citrus limon</i> )	NR	<i>E. coli</i>	>6.4	mg ml <sup>-1</sup>	(Prabuseenivasan et al. 2006)
		<i>S. aureus</i>	>12.8		
Lemongrass ( <i>Cymbopogon citratus</i> )	NR	<i>B. subtilis</i>	>12.8	%	(Naik et al. 2010)
		<i>E. coli</i>	0.06		
		<i>S. aureus</i>	0.03		
		<i>B. cereus</i>	0.03		

**Table 4** (continued)

Common name and scientific name (plant species)	Part of the plant used	Microorganism	MIC	Units	Ref.
Lime ( <i>Citrus aurantium</i> )	NR	<i>Bacillus subtilis</i>	0.06	mg ml <sup>-1</sup>	(Prabuseenivasan et al. 2006)
		<i>Klebsiella pneumoniae</i>	0.25		
		<i>S. aureus</i>	12.8		
		<i>B. subtilis</i>	>6.4		
		<i>K. pneumoniae</i>	>6.4		
		<i>P. vulgaris</i>	>3.2		
		<i>P. aeruginosa</i>	>6.4		
Onion ( <i>Allium cepa</i> )	NR	<i>E. coli</i>	>6.4	mg ml <sup>-1</sup>	(Ye et al. 2013)
		<i>E. coli</i>	0.27		
		<i>B. subtilis</i>	0.18		
		<i>S. aureus</i>	0.18		
		<i>Rhodotorula glutinis</i>	0.36		
		<i>S. cerevisiae</i>	0.36		
		<i>Candida tropicalis</i>	0.36		
		<i>A. niger</i>	0.54		
Orange ( <i>Citrus sinensis</i> )	NR	<i>Monascus purpureus</i>	0.36	mg ml <sup>-1</sup>	(Prabuseenivasan et al. 2006)
		<i>Aspergillus terreus</i>	1.8		
		<i>S. aureus</i>	>12.8		
		<i>B. subtilis</i>	>12.8		
		<i>K. pneumoniae</i>	>12.8		
		<i>P. vulgaris</i>	>6.4		
Oregano ( <i>Origanum vulgare</i> )	Whole plant	<i>P. aeruginosa</i>	>12.8	μg ml <sup>-1</sup>	(Lv et al. 2011)
		<i>E. coli</i>	>12.8		
		<i>Staphylococcus aureus</i>	0.625		
		<i>Bacillus subtilis</i>	0.625		
		<i>Escherichia coli</i>	0.625		
Pennyroyal ( <i>M. pulegium</i> L.)	Aerial parts	<i>Saccharomyces cerevisiae</i>	0.625	μL ml <sup>-1</sup>	(Mahboubi and Haghi 2008)
		<i>Salmonella typhimurium</i>	2.0		
		<i>Staphylococcus aureus</i>	0.5		
		<i>Staphylococcus epidermidis</i>	1.0		
		<i>Escherichia coli</i>	4.0		
		<i>Bacillus cereus</i>	1.0		
		<i>Vibrio cholera</i>	0.5		
		<i>Aspergillus niger</i>	0.25		
		<i>Listeria monocytogenes</i>	1.0		
Perilla ( <i>Perilla arguta</i> )	Leaf	<i>Candida albicans</i>	1.0	μg mL <sup>-1</sup>	(Lv et al. 2011)
		<i>S. aureus</i>	5		
		<i>B. subtilis</i>	2.5		
		<i>E. coli</i>	5		
Rosemary ( <i>Rosmarinus officinalis</i> L.)	Leaves	<i>S. cerevisiae</i>	0.625	μl ml <sup>-1</sup>	(Ojeda-Sana et al. 2013)
		<i>S. aureus</i>	10		
		<i>E. faecalis</i>	25		
		<i>E. coli</i>	14		
	Aerial parts	<i>K. pneumonia</i>	20–40	μL ml <sup>-1</sup>	(Jordán et al. 2013)
		<i>S. aureus</i>	<0.5		
		<i>S. typhimurium</i>	<0.5		
		<i>E. coli</i>	2.0–5.0		
	NR	<i>L. monocytogenes</i>	<0.5	mg ml <sup>-1</sup>	
		<i>S. aureus</i>	>12.8		



**Table 4** (continued)

Common name and scientific name (plant species)	Part of the plant used	Microorganism	MIC	Units	Ref.		
Salvia ( <i>Salvia officinalis</i> L.)	Aerial parts	<i>B. subtilis</i>	>6.4		(Prabuseenivasan et al. 2006)		
		<i>K. pneumonia</i>	>12.8				
		<i>P. vulgaris</i>	>6.4				
				<i>P. aeruginosa</i>	>6.4		
				<i>E. coli</i>	>6.4		
				<i>Ashbiya gossypii</i>	0.125–0.250	mg ml <sup>-1</sup>	(Bouaziz et al. 2009)
				<i>Aspergillus niger</i>	0.125–0.250		
				<i>Bacillus liqueniformis</i>	0.015–0.031		
				<i>C. albicans</i>	0.125–0.250		
				<i>Enterococcus hirae</i>	0.015–0.031		
		<i>Phanerochaetechrysosporium</i>	0.031–0.062				
		<i>Pichia subpelliculosa</i>	0.125–0.250				
		<i>Trichoderma reesei</i>	0.031–0.062				
Salvia ( <i>Salvia officinalis</i> L.)	Aerial parts	<i>E. coli</i>	5.0–10.0	mg ml <sup>-1</sup>	(Longaray Delamare et al. 2007)		
		<i>P. mirabili</i>	5.0–10.0				
		<i>S. typhimurium</i>	5.0–10.0				
		<i>Aeromonas hydrophila</i>	0.5				
		<i>Aeromonas sobria</i>	0.5				
		<i>Klebsiella oxytoca</i>	0.1				
		<i>Citrobacter</i> sp.	5.0–10.0				
		<i>Serratia marcescens</i>	5.0–10.0				
		<i>Bacillus megatherium</i>	0.5				
		<i>B. cereus</i>	0.3				
		<i>B. subtilis</i>	0.4				
		<i>P. aeruginosa</i>	5.0–10.0				
		<i>Pseudomonas fluorescens</i>	5.0				
		<i>S. aureus</i>	5.0–10.0				
		<i>S. epidermidis</i>	5.0–10.0				
		Thyme ( <i>Thymus vulgaris</i> L.)	Aerial parts			<i>S. aureus</i>	1.33
<i>S. epidermidis</i>	1.33						
<i>Streptococcus</i> sp.	2.67						
<i>Pantoea</i> sp.	0.66						
<i>E. coli</i>	0.33–1.33						
NR	<i>Aspergillus</i> sp.		3.2–10.9	μg ml <sup>-1</sup>	(Šegvić Klarić et al. 2007)		
	<i>Penicillium</i> sp.		18.9–19.6				
	<i>Alternaria</i> sp.		2.7–9.4				
	<i>Ulocladium</i> spp.		5.45				
	<i>Absidia</i> spp.		7.0				
		<i>Mucor</i> spp.	50.2				
		<i>Cladosporium</i> sp.	12.8–19.6				
		<i>Trichoderma</i> spp.	16.8				
		<i>Rhizopus</i> spp.	12.6				
		<i>Chaetomium globosum</i>	1.6				
		<i>Stachybotrys chartarum</i>	6.2				

NR, part of the plant used was not reported, MIC minimal inhibitory concentrations

<sup>a</sup> Oil rich in cinnamaldehyde 85.6%

The disk diffusion test, an antimicrobial in vitro evaluation test, is standardized by CLSI (Clinical and Laboratory Standards Institute) and it has been used in several research studies (Hussain et al. 2008; Jordán et al. 2013; Jrah Harzallah et al. 2011; Lv et al. 2011; Melo et al. 2012; Ojeda-Sana et al. 2013). Besides this method, the hole-plate agar diffusion method is another diffusion method that is commonly applied (Bozin et al. 2006; Naik et al. 2010; Al Abbasy et al. 2015). However, researchers adapt experimental methods to have a better representation of the possible future applications of a product or substance in their particular field (Burt 2004). The antimicrobial activity assay can be also carried in broth by the broth microdilution susceptibility assay (Longaray Delamare et al. 2007; Kelen and Tepe 2008).

Antimicrobial assays can be performed in direct contact with the microorganism or by vapor phase. When both methods are compared, the best effectiveness is dependent on the microorganism, the concentration, and type of EO (Fisher and Phillips 2006; Dimić et al. 2014). Lemon EO demonstrated more efficiency in the vapor phase against *Cladosporium cladosporioides* and equal inhibitory effect against *Penicillium chrysogenum* (Dimić et al. 2014).

Viuda-Martos et al. (2011) observed that thyme EO, followed by black cumin EO, presented higher inhibition against *Listeria innocua* and *P. fluorescens* than fennel, parsley, and lavender EO.

Basil (*O. basilicum*) EO did not show inhibition against *Pseudomonas aeruginosa* (Bozin et al. 2006; Al Abbasy et al. 2015; Gaio et al. 2015a), *Staphylococcus epidermidis*, and *Klebsiella pneumonia* (Al Abbasy et al. 2015). On the other hand, Bozin et al. (2006) found a positive antimicrobial effect against *S. epidermidis*, and Melo et al. (2012) showed that the species of basil, *Ocimum micranthum* and *Ocimum selloi*, presented antimicrobial action against *P. aeruginosa*.

Singh et al. (2007) reported an increase in the inhibition zone (measure of the effectiveness of an antimicrobial compound) when the highest volume (6  $\mu\text{L}$ ) of cinnamon EO was used. Cinnamon leaf EO presented a better antifungal (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus ochraceus*, *Aspergillus terreus*, *Fusarium moniliforme*, *Fusarium graminearum*, *Penicillium citrinum*, and *Penicillium viridicatum*) activity than cinnamon bark EO and the opposite was observed for bacteria (*Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *E. coli*, *Salmonella typhi*, *P. aeruginosa*). Cinnamon EO compounds such as E-cinnamaldehyde and eugenol showed a higher antimicrobial activity (Singh et al. 2007) than cinnamic acid (Ooi et al. 2006a).

The antimicrobial activity of sage EO (*Salvia officinalis* L. and *Salvia triloba* L.) against some foodborne microorganisms was proved by Longaray Delamare et al. (2007). These authors found different MIC against *B. cereus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *P. fluorescens*, *Salmonella typhimurium*, and *S. aureus* from that found by Bouaziz

et al. (2009) for *S. officinalis* EO. This discrepancy can be attributed to the bacterial strains and the composition of the EO of different species used in each particular study (Longaray Delamare et al. 2007; Bouaziz et al. 2009).

Gomes Neto et al. (2012) found a high MIC for rosemary EO against *S. aureus*, while Fernández-Pan et al. (2014) found no efficacy for the same oil against this microorganism and observed low inhibition against *L. innocua*, *Salmonella enteritidis*, and *Pseudomonas fragi* (Gomes Neto et al. 2012; Fernández-Pan et al. 2014).

The antifungal and antibacterial activity of the EO from different parts of *Etingera fimbriobracteata* was studied by Ud-Daula et al. (2016). Rhizome oils from *E. fimbriobracteata* showed the highest antibacterial activity, followed by basal stem oils, aerial stem oils, and leaf oils. *E. fimbriobracteata* EO presented a higher antifungal activity against *Saccharomyces cerevisiae* than the antifungal, miconazole (Ud-Daula et al. 2016).

The biological activities of EOs are affected by many factors, such as chemical composition, species, tested microorganisms, method of analysis, method of EO extraction, and other factors, that limit the reproduction of the results. In addition, normalized methods are necessary in order to facilitate the comparison of the results from different authors.

### Essential Oils as Antioxidant Agents

In today's world, with the globalization phenomenon and the technology advances in the food industry, people have access to all foods in every part of the world. In order to make this food availability possible, it is necessary to use an extremely organized and timed transport chain in order to reduce food waste at the lowest levels. Food packaging is designed to protect the nutritional and organoleptic properties of the packaged foods and to make the transport process easier and faster.

One of the problems derived from food degradation is lipid oxidation and peroxidation. These two processes are natural chemical phenomena that affect the nutritional value of foods, and they are responsible for changes in foods flavor, odor, taste, and texture. Lipids, besides contributing to the nutritional value of foods, are essential to a healthy diet and grant organoleptic characteristics to foods (Gutiérrez 2000; Márquez-Ruiz et al. 2008). Foods with high lipid content are highly susceptible to oxidation and peroxidation. In the oxidation process, unsaturated fatty acids interact with the oxygen present in the matrix and in the atmosphere surrounding the food, leading to the formation of peroxide radicals. Once the peroxide radicals are formed, the peroxidation process starts, accelerating the oxidation of lipids (Ferrari 1998; McClements and Decker 2000; Frankel 2005). This natural process can be accelerated in the presence of radiation, oxygen, humidity, high temperature, among other factors (McClements and Decker 2000). Also, the final products of

the lipid oxidation process may lead to cancer, heart disease, cellular mutation, and atherosclerosis (McClements and Decker 2000; Chanwitheesuk et al. 2005; Márquez-Ruiz et al. 2008). This is a major cause of degradation in high-fat content foods, and therefore, it is important to slow down or inhibit this oxidation process. One option is to add additives, such as antioxidants from synthetic or natural origin, directly to food to prevent oxidation, maintaining food quality, and extending the shelf-life of the product.

Antioxidants are compounds capable of slowing down or stop autoxidation of foods, increasing their shelf-life by delaying their natural or induced deterioration (André et al. 2010; Amorati et al. 2013). The most commonly used synthetic antioxidants in food are the BHT (butyl hydroxytoluene) and BHA (butyl hydroxyanisole) (André et al. 2010). The reduction or replacement of synthetic additives for natural additives has encouraged several studies towards the utilization of natural products such as EOs (Kelen and Tepe 2008; Bouaziz et al. 2009; Goñi et al. 2009; Rozman and Jersek 2009). However, the effects of lipid oxidation on proteins, pigments, and lipid degradation can be minimized using natural antioxidants into foods (Li et al. 2014). EOs present a considerable antioxidant capacity as can be seen in Table 5.

The antioxidant capacity of the EOs is directly related to their composition. Phenolic compounds, such as phenolic acids and flavonoids, are considered the major contributors for antioxidant capacity (Choi et al. 2000; Nuutila et al. 2003; Shan et al. 2005; Wei et al. 2014). Their strong antioxidant capacity may be also explained by the synergistic effect among active compounds (Kelen and Tepe 2008).

These compounds act by the inactivation of free radicals and preventing their formation which will avoid the formation of hydroperoxides. Antioxidants can compete with free radicals avoiding the propagation of oxidation reactions (Pokorný et al. 2001; Tian et al. 2013).

An antioxidant can play a completely different role or present a different performance depending on the reactive oxygen species (ROS) or target substrate. Therefore, when selecting the methods to measure antioxidant capacity, the choice of the substrate and its concentration is a very important criterion. For instance, to study the relative bioactivity of an antioxidant or mixture of antioxidants (e.g., plant extract or EO), the application of both aqueous and lipophilic phase systems is important (Patras et al. 2013).

In line with this, antioxidant effect of EOs shall always be evaluated by the combination of at least two or more different *in vitro* antioxidant capacity assays to obtain relevant data regarding their antioxidant activity (Table 4) as suggested by Patras et al. (2013). Antioxidants can act by different mechanisms like the ability to scavenge free radicals, as a hydrogen atom/electron donor or directly reacting with free radicals, and inhibiting the lipid peroxidation (Shahwar et al. 2012; Teixeira et al. 2012).

The antioxidant capacity can be measured using numerous *in vitro* assays such as DPPH<sup>•</sup> (2,2-diphenyl-1-picrylhydrazyl), ABTS<sup>•+</sup> (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), FRAP (ferric-reducing antioxidant power), Rancimat test, ORAC (oxygen-radical antioxidant capacity),  $\beta$ -carotene bleaching, TBARS (thiobarbituric acid reactive species), hydroperoxides, and volatile oxidation products (Amorati et al. 2013).

FRAP, DPPH<sup>•</sup>, and ABTS<sup>•+</sup> methods are the most commonly used (Bernaert et al. 2012; Teixeira et al. 2012; Zeng et al. 2016). DPPH<sup>•</sup> and ABTS<sup>•+</sup> assays measure the scavenging ability of radicals, while FRAP measures the ability to reduce metals, non-radical species such as Fe<sup>3+</sup> ions (Dudonné et al. 2009; Shahwar et al. 2012).

The DPPH<sup>•</sup> method is based in the capacity of a sample to capture the free radical DPPH at room temperature, which causes a decrease in the absorbance at a determined wavelength (515–520 nm) (Brand-Williams et al. 1995; Bondet et al. 1997; Mareček et al. 2017). Initially, the free radical DPPH presents a purple color by having a free electron. When the hydrogen radical is donated to the sample with antioxidant capacity, the purple turns into yellow by the formation of diphenyl-picryl-hydrazine. This method is considered fast and practical and presents a good stability (Arnao et al. 2001). Usually, the free radical DPPH is dissolved in ethanol at concentrations that can vary between 1 and 22.5 mM, but it can also be dissolved in methanol. For instance, in the method described by Moure et al. (2001), the free radical DPPH is diluted in methanol to form a solution of 14.2  $\mu\text{g ml}^{-1}$  (Moure et al. 2001). One of the disadvantages of the free radical DPPH scavenging method is that this method does not represent the reaction that occur naturally in foods. Another disadvantage of this method is that, in the literature, the amount of time that a sample is exposed to the DPPH<sup>•</sup> solution is highly variable, ranging between 20 and 60 or 90 min, the common choice being 30 min (Patras et al. 2013).

The ABTS<sup>•+</sup> method is based on the capture of the ABTS<sup>•+</sup> cation. ABTS<sup>•+</sup> is produced from a precursor, 2,2'-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid, which is oxidized by the potassium persulfate in order to generate the radical. With the addition of an antioxidant, occurs the reduction of the ABTS<sup>•+</sup> which promotes the loss of the coloration of the solution (Rufino et al. 2007). ABTS<sup>•+</sup> is considered stable with high water solubility, and this method is considered fast, practical, and simple (Arnao et al. 2001). However, the low selectivity of ABTS<sup>•+</sup> in the reaction with hydrogen donor atoms presents a disadvantage of this method (Rufino et al. 2007).

On the other hand, the FRAP method is based in the reduction of Fe<sup>3+</sup> to Fe<sup>2+</sup> in the presence of an antioxidant in acid conditions. The formed solution has a blue color with a maximum absorbance at 593 nm (Rufino et al. 2006). This method

**Table 5** Antioxidant activity of essential oils according to different antioxidant tests

Scientific name of the plant used to produce the EO	Part of the plant used	Major compounds (concentration)	Assay				References		
			IC <sub>50</sub> or EC <sub>50</sub> (mg ml <sup>-1</sup> )	DPPH*	ABTS	β-carotene/linoleic acid		Hydroxyl scavenging	Reducing power
<i>Ocimum basilicum</i> (basil)	Aerial parts	Linalool (60.6%)	0.005	–	–	–	–	–	(Hussain et al. 2008)
<i>Thymus vulgaris</i> (thyme)	Leaves, stems and flowers	–	1.10	–	–	–	–	0.80 <sup>b</sup>	(Viuda-Martos et al. 2010)
<i>Origanum vulgare</i> (oregano)	Flowers	–	3.90	–	–	–	–	1.28 <sup>b</sup>	
<i>Syzygium aromaticum</i> (clove)	fruit;	–	0.38	–	–	–	–	1.47 <sup>b</sup>	
<i>Salvia officinalis</i> (salvia)	Leaves and flowers	–	4.20	–	–	–	–	0.12 <sup>b</sup>	
<i>Rosmarinus officinalis</i> (rosemary)	Entire plant	–	17.0	–	–	–	–	0.48 <sup>b</sup>	
<i>Canangaodorata</i> (ylangylang)	Flower	–	0.001	–	–	51.28 <sup>a</sup>	–	–	(Prakash et al. 2012)
<i>Coriandrum sativum</i> (coriander)	Fruits	–	0.003	–	–	25.19 <sup>a</sup>	–	–	
<i>Hedychium spicatum</i> (kapurkacchi)	Rhizome	–	0.022	–	–	8.30 <sup>a</sup>	–	–	
<i>Origanum majorana</i> (marjoram)	Leaves	–	7.2	–	–	22.10 <sup>a</sup>	–	–	
<i>Apiumgraveolens</i> (celery)	Seed	β-Selinene (42.9%)	10.04	–	–	–	–	–	(Teixeira et al. 2013)
<i>Artemisia dracunculus</i> (estragon)	Leaves and flowering tops	Methyl chavicol (92.4%)	8.81	–	–	–	–	–	
<i>Cymbopogon nardus</i> (citronella grass)	Leaves and stems	Δ <sup>2</sup> -Carene (22.5%)	1.18	–	–	–	–	–	
<i>Eugenia</i> spp.	Buds	ρ-Eugenol (67.6%)	0.04	–	–	–	–	–	
<i>Petroselinum sativum</i> (parsley)	Leaves and stems	Myristicin (45.1%)	7.23	–	–	–	–	–	
<i>Thymus capitatus</i> (Mediterranean wild thyme)	Flowering herb	Carvacrol	0.05	–	–	–	–	–	
<i>Thymus vulgaris</i> (thymus)	Flowering	m-Thymol (75.4%)	0.25	–	–	–	–	–	
<i>Artemisia campestris</i> (field seagwort)	Aerial parts	β-Pinene (25.6%)	9.960	1.013	–	–	0.2027	0.305	(Boulanouar et al. 2013)
<i>Rosmarinus officinalis</i> (rosemary)	Leaves	Eucalyptol (18.7%); camphor (15.4%)	0.011	–	–	–	–	–	(Ojeda-Sana et al. 2013)
<i>Curcuma longa</i>	Root	–	5.55 <sup>b</sup>	107.3 <sup>b</sup>	–	–	–	–	

**Table 5** (continued)

Scientific name of the plant used to produce the EO	Part of the plant used	Major compounds (concentration)	Assay				References				
			IC <sub>50</sub> or EC <sub>50</sub> (mg ml <sup>-1</sup> )	DPPH*	ABTS	β-carotene/linoleic acid		Hydroxyl scavenging	Reducing power	FRAP	
(turmeric) <i>Zingiberofficinale</i> (ginger)	Root	–	0.75 <sup>b</sup>	5.23 <sup>b</sup>	–	–	–	–	(Tongnuanchan et al. 2013)		
<i>Zingiber montanum</i> (cassumunar ginger)	Root	–	8.71 <sup>b</sup>	54.7 <sup>b</sup>	–	–	–	–	–		
<i>Thymus algeriensis</i> (thymus)	Aerial parts	Thymol (56%)	1.64	–	1.56	–	–	0.68	–	(Nikolic et al. 2014)	
<i>Eucalyptus globules</i> (Tasmanian blue gum)	Leaves	Eucalyptol (55.29%)	33.33	–	6.753	–	–	115.39	–	(Harkat-Madouri et al. 2015)	
<i>Hertia cheirifolia</i>	Flower	α-Pinene (70.4%)	0.016	–	0.034	–	–	0.021	–	(Majouli et al. 2016)	
<i>Salvia officinalis</i> (sage)	Leaves and flowers	–	1.999	–	–	–	–	–	–	(Adrar et al. 2016)	
<i>Thymus numidicus</i> (thymus)	Leaves and flowers	–	0.156	–	–	–	–	–	–	–	
<i>Vitex agnus-castus</i> (vitex)	Seeds	Eucalyptol (19.61%); sabinene (14.57%)	1.072	–	–	–	–	–	–	–	(Asdadi et al. 2015)
<i>Coriandrum sativum</i> (coriander)	–	Linalool (64.38%)	5.84 <sup>a</sup>	–	0.23 <sup>a</sup>	–	–	–	–	–	(Duarte et al. 2016)
<i>Etilingera fimbriobracteata</i> (layun)	Rhizomes	Decanal (34.4%)	228.3	11.22	–	–	–	57.61	1.01	–	(Ud-Daula et al. 2016)
<i>Eucalyptus globules</i> (Tasmanian blue gum)	Leaves and branches	Eucalyptol (63.81%)	2.90 <sup>a</sup>	–	2.72 <sup>a</sup>	–	–	–	–	–	(Luis et al. 2016)
<i>Illicium verum</i> (star anise)	Fruit	Trans-anethole	0.047	0.076	–	–	–	–	–	–	(Asif et al. 2016)
<i>Mentha pulegium</i> (pennyroyal)	Leaves	Pulegone (70.4%)	–	0.057	–	–	–	–	–	–	(Brahmi et al. 2016)

IC<sub>50</sub> and EC<sub>50</sub> (mg ml<sup>-1</sup>): effective concentration that inhibits 50% of the radical  
 DPPH 2,2-difenil-1-picril-hidrazil, ABTS 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), FRAP ferric-reducing antioxidant power

<sup>a</sup> Results in percentage (%)

<sup>b</sup> Results in mMol Trolox equivalent/l

is considered fast and reproducible; however, besides being unable to measure all the antioxidants present in a complex food matrix, it requires an aqueous system, and it does not detect compounds that act by the donation of hydrogen atoms and can be reduced by other compounds leading to false results (Magalhães et al. 2008).

The Rancimat test, due to its easy application and reproducibility, is also a widely applied method that measures the oxidative stability of fats and oils (García-Moreno et al. 2013). The method is based on the measurement of the volatile products of the oxidation that are dissolved in distilled water (Velasco et al. 2009). In this assay, the sample of fat or oil is passed through a stream of purified air. Once the air contains the volatile organic acids produced during the oxidation of the oil or the fat sample, the air is used to oxygenize the deionized water, whose conductivity is being constantly monitored. The conductivity of the water, once mixed with the air, increases (AOCS 1994).

Boulanouar et al. (2013) compared the antioxidant capacity of *Artemisia campestris* L. EO by several methods such as TBARS, lecithin liposome oxidation assay, ferrous ion-chelating ability assay, and 5-lipoxygenase inhibition assay. The results expressed as  $IC_{50}$  ( $mg\ mL^{-1}$ ) (concentration of extract able to prevent 50% lipid oxidation) were 0.849, 0.080, 0.182, and 0.090, respectively (Boulanouar et al. 2013).

ORAC method with results expressed as  $gTrolox/gEO$  ( $gT/gEO$ ) was the selected method to analyze the antioxidant capacity of EOs by Bentayeb et al. (2014). Oregano (2.62  $gT/gEO$ ), clove (2.43  $gT/gEO$ ), and cinnamon (2.11  $gT/gEO$ ) EOs showed the highest antioxidant capacity, followed by red thyme (1.24  $gT/gEO$ ) and thyme (0.79  $gT/gEO$ ) EOs (Bentayeb et al. 2014).

EOs can present the same or higher antioxidant capacity than synthetic antioxidants. Bouaziz et al. (2009) demonstrated that sage EO exhibited a remarkably higher antioxidant capacity than BHT (Bouaziz et al. 2009). While *Hertiacheirifolia* L. EO from flowers showed a lower antioxidant capacity than BHT regarding DPPH<sup>•</sup> and  $\beta$ -carotene bleaching tests (Majouli et al. 2016).

Sage and rosemary EOs showed the highest antioxidant capacity according to DPPH<sup>•</sup>, TBARS, FRAP, and Rancimat tests, while clove and oregano EOs showed the lowest antioxidant capacity for the first three tests, followed by thyme. However, in Rancimat test, oregano and thyme had the highest antioxidant capacity. Clove presented a higher antioxidant capacity than BHT and ascorbic acid in the DPPH<sup>•</sup> and FRAP assays. In the ferrous iron chelating assay, all EOs presented better antioxidant capacity than BHT, and ascorbic acid, rosemary, and sage EOs were the most efficient and oregano showed the lowest antioxidant capacity (Viuda-Martos et al. 2010).

According to Singh et al. (2007), depending on the plant and the part of the plant used to produce the EO, the antioxidant capacity can vary considerably. Besides, cinnamon bark EO presented a higher antioxidant capacity in the DPPH<sup>•</sup> and hydroxyl<sup>•</sup> radical assays than cinnamon leaf EO. Among the

constituents of cinnamon EO, an amount of 25  $\mu L$  of eugenol showed a higher radical scavenging activity by DPPH<sup>•</sup> assay (92.9%) than the same amount of E-cinnamaldehyde (78.3%). It is possible that the radical scavenging activity might be mostly affected by the position of the phenolic hydroxyl group which is present in eugenol (Singh et al. 2007).

In the DPPH<sup>•</sup> assay, the free radical inhibition (%) for rosemary EO was determined to be 62.45%, whereas the values of their major constituents were 42.74%, 45.61%, and 46.21% ( $v/v$ ) for 1,8-cineole,  $\alpha$ -pinene, and  $\beta$ -pinene, respectively. In the  $\beta$ -carotene bleaching test, the concentrations providing 50% inhibition ( $IC_{50}$ ) were 2.04, 4.05, 2.28, and 2.56% ( $v/v$ ) for the EO, 1,8-cineole,  $\alpha$ -pinene, and  $\beta$ -pinene, respectively (Wang et al. 2008). This indicates that minor components contributed to the antioxidant capacity of rosemary EO.

The different units of expressing the results of the same method can complicate the comparison among EOs' antioxidant capacity. There is a need for a standardized approach in order to generate meaningful data that can be compared among different sources (Amorati et al. 2013). According to Patras et al. (2013), expressing kinetic parameters as  $EC_{50}$  provides a more comprehensive evaluation of antioxidant activity.

The broad variety of potential antioxidant compounds can result in a wide variation of the antioxidant capacity of the EOs (Nuutila et al. 2003; Viuda-Martos et al. 2010).

## Determination of Active Compounds: Analysis Methods

In order to be able to study the EOs, it is important to evaluate their components, but this can become a difficult task. Besides the numerous compounds present in each EO, most of them are present in minor quantities requiring methods with low detection limits. The separation of the compounds can be achieved by fractional distillation, GC, high-performance liquid chromatography (HPLC), MS, and nuclear magnetic resonance (NMR) spectroscopy (Nakatsu et al. 2000; Tisserand and Young 2014).

Fractional distillation is used to purify the EOs or to concentrate the fraction or fractions of the EO to be further evaluated. The main goal is to separate substances through the volatility difference among them. Apart from depending of the natural physico-chemical properties of the substance to be analyzed, it also depends on the pressure and temperature of the system. Generally, this method is used as pre-treatment or pre-purification of the sample and cannot achieve the necessary separation resolution to the study of the biological activities of EOs (Nakatsu et al. 2000; Silvestre et al. 2016). Chromatography is the most used technique when it comes to chemical separation of EO components. Several conditions used to quantify and identify the compounds present in the EO

**Table 6** Some essential oils and their major compounds analyzed by chromatography

Essential oil of	Major components	Method/ detector	Conditions	Analytical column	Ref.
<i>Agastache rugosa</i> (flower)	Pulegone (34.1%), estragole (29.5%) and <i>p</i> -menthan-3-one (19.2%)	GC-FID and MS	Injection temperature (FID/MS): 250 °C Detector temperature (FID/MS): 250 °C Injection mode (MS): split Oven ramp (FID/MS): from 40 °C to 250 °C (5 °C/min)	HP-5 (30 m × 0.32 mm; film thickness 0.25 μm)	(Haiyan et al. 2016)
<i>Agastache rugosa</i> (leaf)	<i>p</i> -Menthan-3-one (48.8%) and estragole (20.8%).		Carrier gas (FID/MS): helium Injection volume (MS): 0.2 μl		
<i>Alpinia zerumbet</i> leaves	1,8-Cineol (16.63%), camphor (14.1%), methyl cinnamate (12.81%) and borneol (6.41%)	HPLC-UV and GC-MS	HPLC-UV: Mobile phase: (solvent A) water with 1% acetic acid (v/v) and (solvent B) methanol:acetonitrile:acetic acid (95:4:1, v/v/v) Flow: 0.8 ml/min Mobile phase gradient: from 0 to 2 min, 5% B isocratic; 2–10 min, linear gradient 5–25% B; 10–20 min, linear gradient 25–40% B; 20–30 min, linear gradient 40–50% B; 30–40 min, linear gradient 50–100% B; 40–45 min, 100% B isocratic and 45–55 min, linear gradient 100–5% B.	HPLC-UV column: RP-18 ZORBAX ODS column (2.5 × 0.46 cm; 5 μm particle size) GC-MS column: DB-5MS column (30 m × 0.25 mm, 0.25-μm film thickness)	(Elzaawely et al. 2007)
<i>Alpinia zerumbet</i> seeds	α-Cadinol (13.46%), <i>T</i> -muurolol (10.79%), α-terpineol (10.67%), δ-cadinene (6.19%) and terpinene-4-ol (6.18%)		GC-MS Injector temperature: 250 °C Detector temperature: 280 °C Oven ramp: from 40 °C (5 min) to 280 °C (6 °C/min) and hold for 5 min		
Basil ( <i>Ocimum gratissimum</i> )	Eugenol (67.1%)	GC-MS and FID	Carrier gas: helium Injector temperature: 250 °C Detector temperature: 250 °C Injection mode: split Oven ramp: from 70 to 180 °C at 4 °C/min to 250 °C at 10 °C/min	Non-polar VF-5MS-fused silica capillary column (30 m × 0.25 mm, 0.25-μm film thickness)	(Bastos et al. 2016)
Caraway ( <i>Carum carvi</i> )	Carvone (52.22%) and limonene (43.53%)	GC-FID	Carrier gas (MS): helium Carrier gas (FID): hydrogen Injector temperature: 220 °C Injector detector: 260 °C Injection mode: split Oven ramp: from 60 °C for 2 min, to 220 °C (4 °C/min) and isotherm at 220 °C for 5 min	HP 20 M column (25 m, 0.32 mm, 0.3-μm film thickness)	(Gniewosz et al. 2013)
Cinnamon ( <i>Cinnamomum verum</i> )	Eugenol (3.402 g/ml), cinnamaldehyde (0.652 g/ml) and acetoeugenol.	GC-MS	Carrier gas: helium Injector temperature: 100 °C Injector detector: 225 °C Oven ramp: from 55 °C held for 1 min, to 95 °C (3 °C/min), to 220 °C (25 °C/min), held for 10 min	AT-1 capillary column (30 m × 0.25 mm, 0.25-μm film thickness)	(Avila-Sosa et al. 2012)
		GC-MS	Carrier gas: helium Injector temperature: 250 °C		(Pires et al. 2013)

Table 6 (continued)

Essential oil of	Major components	Method/ detector	Conditions	Analytical column	Ref.
Citronella ( <i>Pelargonium citrosum</i> )	Sesquiterpenes hydrocarbons (38.3%) ( $\beta$ -elemene, $\delta$ -cadinene and eremophilene), monoterpene hydrocarbons (27.6%) ( $\Delta^3$ -carene and $\gamma$ -terpinene) and (22.5%) Oxygenated Monoterpenes ( $\beta$ -citronellal and $\beta$ -citronellol)		Injection mode: split Carrier gas: helium	Fused silica capillary column HP-5MS, 5% diphenyl 95% dimethyl polydimethylsiloxane (30 m $\times$ 0.32 mm, 0.25- $\mu$ m film thickness)	(Aguilar-González et al. 2015)
Clove ( <i>Syzygium aromaticum</i> )	Eugenol (75.39%), caryophyllene oxide (11.22%) and eugenol acetate (5.16%)	GC-MS	Injector temperature: 250 °C Injection mode: split Oven ramp: from 60 °C/2 min, to 250 °C (10 °C/min), held for 10 min Carrier gas: helium Oven ramp: 50 to 300 °C (15 min)	Agilent HP-5 column (30 cm $\times$ 0.25 mm, 0.25- $\mu$ m film thickness)	(Morelli et al. 2015)
Copaifera multijuga	Copaene, $\beta$ -gurjunene, $\beta$ -caryophyllene, $\beta$ -cubebene, $\beta$ -bisabolene and $\delta$ -cadinene	GC-MS	Injection mode: split Carrier gas: helium	HP-5MS 5% phenylmethyl siloxane column	(Pires et al. 2013)
Coriander ( <i>Coriandrum sativum</i> )	Monoterpene hydrocarbons ( $\Delta^3$ -carene and $\gamma$ -terpinene) (73.2%)	GC-MS	Injection mode: split Carrier gas: helium	Fused silica capillary column HP-5MS, 5% diphenyl 95% dimethyl polydimethylsiloxane (30 m $\times$ 0.32 mm, 0.25- $\mu$ m film thickness)	(Pilar Santamarina et al. 2016)
(Dried bay leaf) <i>Laurus nobilis</i> L.	1,8-Cineole (51.95%), $\alpha$ -terpinyl acetate (12.93%) and monoterpene hydrocarbon sabinene (9.56%)	GC-MS	Injection mode: split Injector temperature: 220 °C Detector temperature: 250 °C Oven ramp: from 60 (5 min) to 180 (3 °C/min) to 280 °C (20 °C/min) for 10 min Carrier gas: helium	VA-5MS capillary column (30 m $\times$ 0.25 mm, 0.25- $\mu$ m film thickness)	(Pilar Santamarina et al. 2016)
Lavender ( <i>L. officinalis</i> L.)	Linalool (53.50%)	GC-FID	Carrier gas: helium Injector temperature: 220 °C Detector temperature: 250 °C Oven ramp: from 60 (5 min) to 180 (3 °C/min) to 280 °C (20 °C/min) for 10 min Carrier gas: helium	HP-1 (cross-linked methyl silicone) capillary column (30 m $\times$ 0.2 mm, 0.33- $\mu$ m film thickness)	(Martucci et al. 2015)
Lemon	Limonene (62.12%), $\beta$ -pinene (14.34%), $\gamma$ -terpinene (8.88%)	GC-MS	Injection mode: split Injector temperature: 280 °C Injection volume: 1 $\mu$ l Oven ramp: from 50 (2 min) to 260 °C (10 °C/min) and then hold for 2 min Carrier gas (MS): helium Injector temperature: 250 °C Injector detector: 250 °C Injection mode: split Injection volume: 0.3 $\mu$ l Oven ramp: from 70 °C for 10 min to 120 °C (3 °C/min), to 220 °C (4 °C/min), to 280 °C (15 °C/min) during 10 min Carrier gas: helium	Agilent 5MS (100 m $\times$ 0.25 mm, 0.25- $\mu$ m thickness)	(Volpe et al. 2015)
Lemongrass ( <i>Cymbopogon citratius</i> )	Citral (84.0%)	GC-MS and FID	Injector temperature: 250 °C Detector temperature: 250 °C Injection mode: split Oven ramp: from 70 to 180 °C at 4 °C/min to 250 °C at 10 °C/min	DB-5 (Restek Co.) column (30 m $\times$ 0.25 mm, 0.25- $\mu$ m film thickness)	(Bastos et al. 2016)



Table 6 (continued)

Essential oil of	Major components	Method/ detector	Conditions	Analytical column	Ref.
	Geranial (2.873 g/ml)	CG-MS	Carrier gas (MS): helium Carrier gas (FID): hydrogen Injector temperature: 100 °C Injector detector: 225 °C Oven ramp: from 55 °C held for 1 min, to 95 °C (3 °C/min), to 220 °C/25 °C/min, held for 10 min	AT-1 capillary column (30 m × 0.25 mm, 0.25- $\mu$ m film thickness)	(Avila-Sosa et al. 2012)
<i>Lippia gracilis</i> Schauer	Thymol (59.26%) and carvacrol (43.24%)	CG-MS	Carrier gas: helium Injector temperature: 250 °C Injector detector: 280 °C Injection volume: 0.5 $\mu$ l Oven ramp: from 50 °C for 1.5 min, to 200 °C (4 °C/min), to 250 °C (15 °C/min) during 5 min. Carrier gas: helium	Fused silica capillary column (5%-phenyle-95%--dimethylpolysiloxane, 30 m × 0.25 mm, 0.25- $\mu$ m film thickness)	(Azevedo et al. 2014)
Apple mint ( <i>Mentha suaveolens</i> spp. <i>insularis</i> )	Pulegone (46.52%)	CG-MS and FID	Head pressure: 64.20 kPa Injector temperature: 250 °C Detector temperature: 250 °C Injection mode: splitOven ramp: from 3 min at 60 °C (isothermal) to 210 °C (4 °C/min) for 15 min to 300 °C (10 °C/min) for 15 min Carrier gas: helium	HP-5 capillary (30 m × 0.25 mm, 0.17- $\mu$ m film thickness)	(Petretto et al. 2014)
Mexican lime bagasse	Limonene, $\alpha$ -pinene, $\beta$ -pinene, $\alpha$ -terpineol and Myrcene	CG-MS	Injector temperature: 180 °C Detector temperature: 180 °C Oven ramp: from 60 °C for 0.5 min to 228 °C (3 °C/min)	Non-polar PE-5 (5% phenyl-methyl-silicone) (60 m × 0.25 mm, 1- $\mu$ m film thickness)	(Sánchez Aldana et al. 2015)
Mexican oregano ( <i>Lippia berlandieri</i> Schauer)	Thymol (2.103 g/ml) and carvacrol (0.533 g/ml)	CG-MS	Carrier gas (MS): helium Injector temperature: 100 °C Injector detector: 225 °C Oven ramp: from 55 °C held for 1 min, to 95 °C (3 °C/min), to 220 °C/25 °C/min, held for 10 min carrier gas: helium	AT-1 capillary column (30 m × 0.25 mm, 0.25- $\mu$ m film thickness)	(Avila-Sosa et al. 2012)
Mustard ( <i>Brassica nigra</i> )	Allyl isothiocyanate (98.42%)	CG-MS	Injector temperature: 250 °C Injection mode: split Oven ramp: from 60 °C/2 min, to 250 °C (10 °C/min), held for 10 min Carrier gas: helium	Agilent HP-5 column (30 cm × 0.25 mm, 0.25- $\mu$ m film thickness)	(Aguilar-González et al. 2015)
Oregano	Carvacrol	CG-FID	Carrier gas: helium Injector temperature: 250 °C Injection mode: split Injection volume: 1 $\mu$ l Oven ramp: 80–120 °C at 5 °C/min Carrier gas: nitrogen	DB-5 capillary column (30 m × 0.32 mm, 0.50- $\mu$ m film thickness)	(Muriel-Galet et al. 2015)
Oregano ( <i>O. vulgare</i> L.)	Carvacrol (81.85%) $\alpha$ -Methyl cinnamaldehyde, thymol, p-cimene, caryphyllene	CG-MS		HP-5MS (30 m × 0.25 mm, 0.25- $\mu$ m film thickness) Low polar-fused silica (5% phenylmethylpolysiloxane) capillary	(Hosseini et al. 2016) (Llana-Ruiz-Cabello et al. 2015)

Table 6 (continued)

Essential oil of	Major components	Method/ detector	Conditions	Analytical column	Ref.
Rosemary pepper ( <i>Lippia sidoides</i> )	Carvacrol (26.70%), p-cimene (15.20%) and $\gamma$ -terpinene	CG-MS	Oven ramp: from 50 °C (1 min) to 100 °C (30 °C/min), to 300 °C (10 °C/min) and stabilized at 300 °C for 10 min Carrier gas: helium Injector temperature: 280 °C Injection mode: split Injection volume: 1 $\mu$ l Oven ramp: from 50 °C (2 min), to 260 °C (10 °C/min) and then hold for 2 min Carrier gas (MS): helium Injector temperature: 170 °C Injector detector: 170 °C	column J&W HP-5MS Ultra Inert, (30 m $\times$ 25- $\mu$ m, 0.25- $\mu$ m film thickness) 100 m $\times$ 0.25 mm, 0.25- $\mu$ m film thickness	(Martucci et al. 2015)
Oregano ( <i>Origanum spp.</i> )	$\gamma$ -Terpinene and p-cimene	CG-MS	Carrier gas (MS): helium Injector temperature: 170 °C Injector detector: 170 °C	DB-WAX (60 m $\times$ 0.25 mm)	(Botre et al. 2010)
Rosemary pepper ( <i>Lippia sidoides</i> )	Thymol (52.7%)	CG-MS and FID	Oven ramp: from 60 (4 min) to 170 °C (5 °C/min). Injector temperature: 250 °C Detector temperature: 250 °C Injection mode: split Oven ramp: from 70 to 180 °C at 4 °C/min to 250 °C at 10 °C/min Carrier gas (MS): helium Carrier gas (FID): hydrogen	Non-polar VF-5MS-fused silica capillary column (30 m $\times$ 0.25 mm, 0.25- $\mu$ m film thickness)	(Bastos et al. 2016)
Clove leaf ( <i>Syzygium aromaticum L.</i> )	Phenylpropanoid eugenol (88.58%), $\beta$ -caryophyllene (8.13%) and $\alpha$ -humulene (2.35%)	CG-MS	Injection mode: split Injector temperature: 220 °C Detector temperature: 250 °C Oven ramp: from 60 °C (5 min), to 180 °C (3 °C/min), to 280 °C (20 °C/min) for 10 min Carrier gas: helium	VA-5MS capillary column (30 m $\times$ 0.25 mm, 0.25- $\mu$ m film thickness)	(Pilar Santamarina et al. 2016)
Tarragon ( <i>Artemisia dracunculoides</i> )	Methyl chavicol (92.4%)	CG-FID	Injector temperature: 220 °C Detector temperature: 250 °C Oven ramp: from 60 °C (5 min), to 180 °C (3 °C/min), to 280 °C (20 °C/min) for 10 min. Carrier gas: helium	Capillary column (30 m $\times$ 0.2 mm, 0.33- $\mu$ m film thickness)	
<i>Tetrachinis articulata</i> (Vahl.) Masters.	$\alpha$ -Pinene (56.21%), 1,8-cineole (9.91%), isobronyl acetate (7.46%), $\beta$ -myrcene (3.08), $\delta$ -gurjuene (2.45%)	CG-MS and FID	Injection mode: split Injector temperature: 250 °C Detector temperature: 280 °C Injection volume: 0.1 ml Oven ramp: from 50 °C (1 min), to 240 °C (5 °C/min) and held isothermal for 4 min Carrier gas: nitrogen	Fused silica capillary column HP-5MS, 5% diphenyl 95% dimethyl polydimethylsiloxane (30 m, 0.32 mm, 0.25- $\mu$ m film thickness) Fused silica capillary column, apolar HP-5 (30 m $\times$ 25 mm, 0.25- $\mu$ m film thickness).	(Pires et al. 2013) (Ben Ghnaya et al. 2016)
Thyme ( <i>Thymus vulgaris</i> )	Thymol (75.4%) and carvacrol (5.4%)	CG-MS	Injection mode: split Injector temperature: 250 °C Detector temperature: 280 °C Injection volume: 0.1 ml Oven ramp: from 50 °C (1 min), to 240 °C (5 °C/min) and held isothermal for 4 min Carrier gas: nitrogen	Fused silica capillary column HP-5MS, 5% diphenyl 95% dimethyl polydimethylsiloxane	(Pires et al. 2013)

**Table 6** (continued)

Essential oil of	Major components	Method/ detector	Conditions	Analytical column	Ref.
<i>Zataria multiflora</i> Boiss.	Thymol (65.8%) and carvacrol (6.1%)	HPLC-UV	Mobile phase: acetonitrile: distilled water (40:60 v/v) Injection Volume: 10 µl Flow: 1.0 ml/min	(30 m × 0.32 mm, 0.25-µm film thickness) Kromasil 100-5C18	(Ansorena et al. 2016)
	Carvacrol (41.2%) and thymol (27.4%)	CG-MS	Oven ramp: from 50 °C to 120 °C (2 °C/min), held for 3 min at this temperature, to 300 °C Carrier gas: helium	HP-5MS capillary column (30 m × 0.25 mm, 0.25-mm film thickness)	(Raecisi et al. 2015)

**Table 7** *Ferula assa-foetida* L. EO from nine locations and their major compounds analyzed by gas chromatography

Essential oil	Major components	Method/ detector	Conditions	Analytical column	Ref.	
<i>Ferula assa-foetida</i> L.	(E)-Propenyl sec-butyl disulfide	FID and MS	n-Propyl sec-butyl disulfide 0% 4.07% 0% 3.57% 3.73% 2.46% 5.01% 3.95% 3.95%	FID column: DB-5 fused silica column (30 m × 0.25 mm, 0.25-µm film thickness) MS Column: DB-5 fused silica column (60 m × 0.25 mm, 0.25-µm film thickness)	(Moghaddam and Farhadi 2015)	
	(E)-Propenyl sec-butyl disulfide					4.72%
	β-Pinene					9.66%
	(E)-β-Ocimene					5.50%
	α-Pinene					4.96%
	(E)-β-Pinene					10.69%
	(Z)-Propenyl sec-butyl disulfide					23.10%
	(Z)-Propenyl sec-butyl disulfide					22.10%
	(Z)-Propenyl sec-butyl disulfide					22.80%
Loc. 1	40.41%	250 °C	Detector temperature: 280 °C			
Loc. 2	40.31%	Injection mode: split	Oven ramp: from 60 to 250 °C (4 °C/min) and held isothermal at 250 °C for 10 min			
Loc. 3	44.39%	Carrier gas: nitrogen	MS conditions: Oven ramp: from 60 °C to 250 °C (4 °C/min) and held isothermal at 250 °C for 10 min			
Loc. 4	50.03%	Carrier gas: helium				
Loc. 5	49.09%					
Loc. 6	37.30%					
Loc. 7	42.62%					
Loc. 8	52.24%					
Loc. 9	53.99%					

Loc. localization



**Table 9** Toxicity of essential oils and their constituents (adapted from Raut and Karuppaiyil 2014)

Essential oils or their constituents	Toxicity
<i>Mentha pulegium</i>	Internal bleeding and damage to lungs
<i>Mentha pulegium</i> (containing pulegone and menthofuran)	Hepatotoxic effect
Estragol	Carcinogenic effect
Methyl iso-eugenol	Carcinogenic effect
Limonene	Hepatotoxic, nephrotoxic, carcinogenic, foetotoxic, and teratogenic effects. Diarrhea and transient proteinuria
Citral	Histological cell necrosis and vacuolization
<i>Eucalyptus globulus</i> (containing 1,8-cineole);	Convulsions, hepatic necrosis, dementia, ataxia, and hallucinations.
<i>Foeniculum vulgare</i> (containing fenchone);	
<i>Mentha pulegium</i> (containing pulegone);	
<i>Rosmarinus officinalis</i> (containing camphor);	
<i>Mentha</i> sp. (containing menthol and menthone);	
<i>Artemisia absinthium</i> (containing thujone).	
<i>Salvia. aromaticum</i> ,	Act as irritants
<i>Coriander sativum</i> (containing Linalool);	
<i>Melissa officinalis</i> ;	
<i>Origanum vulgare</i> ;	
<i>Satureja hortensis</i> ;	
<i>Melaleuca alternifolia</i> ;	
<i>Thymus vulgaris</i> ;	
<i>Pinus sylvestris</i>	
<i>Citrus aurantium</i> ;	Induce phototoxicity
<i>Cuminum cyminum</i> ;	
<i>Citrus paradise</i> ;	
<i>Citrus limon</i> ;	
<i>Citrus sinensis</i>	
<i>Anethum graveolens</i> ;	Toxic during pregnancy and may have abortifacient effects
<i>Juniperus sabina</i> ;	
<i>Artemisia absinthium</i> ;	
<i>Pimpinellaanisum</i> (containing anethole);	
<i>Foeniculum vulgare</i> (containing anethole);	
<i>Myristicafragrans</i> (containing safrole and myristicin);	
<i>Rosmarinus officinalis</i> (containing camphor).	
<i>Acoruscalamus</i> (containing asarone);	Carcinogenic effect
<i>Croton tiglium</i> ;	
<i>Ocimum</i> spp.;	
<i>Myristicafragrans</i> ;	
<i>Rosa</i> spp. (containing >3.0% of methyl eugenol)	
Citral;	Induce cytotoxicity and genotoxicity in human lymphocytes at higher concentrations
Geraniol	
<i>Cymbopogon martini</i> ;	

**Table 9** (continued)

Essential oils or their constituents	Toxicity
<i>Cymbopogon citratus</i> ; <i>Chrysopogonizanioides</i> <i>Artemisia absinthium</i> (containing thujone); <i>Mentha pulegium</i> (containing pulegone); <i>Acoruscalamus</i> (containing asarone); <i>Brassica nigra</i> (containing allyl isocyanate)	Induce cytotoxicity and genotoxicity in human lymphocytes at higher concentrations Toxic effects in humans

Most toxic effects were observed in animals

between the effective EO dose and the risk of toxicity (Sánchez-González et al. 2010), since some EOs are found to be safe for human consumption at low concentrations (Raut and Karuppaiyl 2014).

Toxicity of EO depends on the frequency of use, on the susceptibility of the individual to potentially toxic substances which can vary considerably, and also on the concentrations used (Unlu et al. 2010; Raut and Karuppaiyl 2014). EO interactions can occur between one and more of their constituents, as well as between a component and drug or a food item (Unlu et al. 2010). Table 9 reports some toxic effects of the EOs or their constituents. Most of the toxic effects were observed in animal studies (adapted from Raut and Karuppaiyl 2014).

EOs may cause allergic reactions, such as dermatitis by their frequent use, as it happens in aromatherapy (Bleasel et al. 2002; Trattner et al. 2008). Various uncovered parts of the skin, in particular the scalp, neck, and hands, can be affected. Lavender, jasmine, rosewood, laurel, eucalyptus, and pomegranate EOs have shown some allergic effects (Schaller and Korting 1995). Data of Information Network of Departments of Dermatology (IVDK) have reported a total of 637 cases of allergy to ylang-ylang, lemongrass, jasmine, sandalwood, and clove EOs in Germany between 2000 and 2008 (Uter et al. 2010).

A study carried out by Groot and Schmidt (2016) reported that nearly 80 EOs have caused contact allergy. In general, most of the reactions were caused by application of pure oils or high-concentration products.

Sometimes only a single major component isolated from the EO is analyzed, as in the study carried out by Audrain et al. (2014). In this study, limonene, which is constituent of the lime and orange EOs, and linalool, which is constituent of the basil and lavender EOs, have been associated with allergic contact dermatitis (Audrain et al. 2014).

## Concluding Remarks

The deterioration by microorganisms and oxidation of lipids are the important causes of food loss. Therefore, control of the

growth of microorganisms and lipid oxidation are necessary to increase food stability and to extend product shelf-life and consequently food safety and industry economic profitability.

Microbial and antioxidant in vitro assays were reported as analysis techniques suitable to study the potential biological activities of EOs. In fact, diffusion in agar and DPPH<sup>•</sup> assay were more frequently used assays to evaluate antimicrobial and antioxidant capacity, respectively. In general, volatile active agents of EOs were determined by gas chromatography. The identification and quantification of EO constituents are of great importance in order to assure their appropriate use. It was shown that most of the EOs have antimicrobial, antioxidant, or both properties, and their composition of EOs has generally wide variability, which greatly influences their biological activity. Therefore, the standardization of the EO manufacturing process is a way of ensuring their effectiveness and safety. Moreover, in spite of the potential of EOs, more studies such as clinical trials should evaluate their safety and possible side effects before considering their use for food or food packaging purposes.

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