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# Role of Natural Essential Oils in Sustainable Agriculture and Food Preservation

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## **Author's contribution**

*This whole work was carried out by the author MAB.*

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## **ABSTRACT**

Research on humans, animals or plants about anti-inflammatory, anticancer, antiviral, repellent, antibacterial, antifungal or antioxidant activities of the essential oils corroborated the biological characteristics of aromatic plants and their use since ancient times for their preservative and medicinal properties. These mixtures of natural compounds are valuable ingredients in perfumery, food, agricultural and pharmaceutical industries. Currently, consumer demand natural products, effective, safe and environmentally friendly. Among them, essential oils may be natural alternatives of synthetic herbicides for organic farming systems, solving serious environmental problems due to their low persistence in the field as well as the incidence of resistance in both weeds and some pathogens. Correlations between the principal compounds of essential oils with herbicidal effect than explain their use in a sustainable agriculture or their antibacterial activity against food borne pathogens, food spoiling bacteria and bacterial virulence factors as biofilm formation for the use as natural food preservative are the main focus of this review.

*Keywords: Essential oil; herbicidal activity; antimicrobial activity; food preservatives.*

## **1. INTRODUCTION**

Essential oils, complex oil mixture easily and economically obtained by steam distillation from different parts of aromatic plants or by cold-presson (*Citrus* fruits), are widely used in

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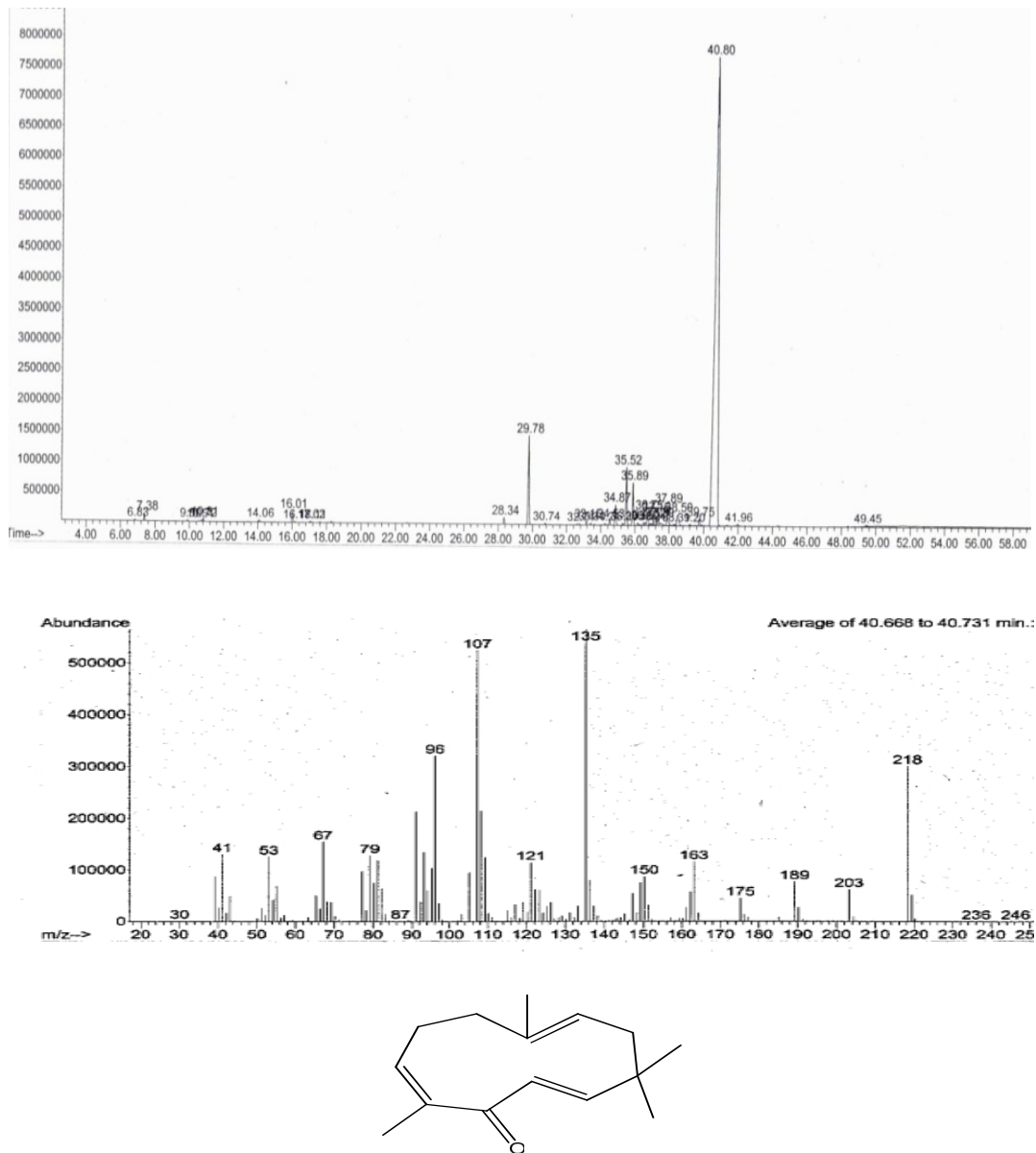
perfumery, cosmetic, food, beverage, agricultural and pharmaceutical industries. Several studies have demonstrated that depending on its composition, certain essential oils are responsible of a large number of pharmacological activities such as expectorant (e.g. eucalyptus essential oil) rubefacient (e.g. turpentine and rosemary essential oils), carminative, eupeptic, antispasmodic (e.g. chamomile and mint essential oils), antiseptic (e.g. thyme, oregano essential oils), antibacterial (e.g. eucalyptus and thyme essential oils), anti-inflammatory (e.g. chamomile essential oil,) etc. It is well known that carminative, eupeptic and antispasmodic properties are related with essential oils with anethol; rubefacient property are attributed to essential oils rich in camphor or antiseptic activity are related with essential oils with high content in phenolic compounds such as thymol or carvacrol. Recent investigations corroborated that the anti-inflammatory activity of chamomile essential and their use in inflammatory disorders of the gastrointestinal tract [1] is due to both sesquiterpene compounds,  $\alpha$ -bisabolol and chamazulene. In response to carrageenan, the oxygenated sesquiterpene  $\alpha$ -bisabolol decreased leukocyte migration, protein extravasations and the amount of TNF- $\alpha$  in rat peritoneal cavity [2], whereas the sesquiterpene hydrocarbon chamazulene, was able to suppress the formation of leukotiene B<sub>4</sub> in the neutrophilic granulocytes [3]. In the last years a wide range of promising biological activities, such as herbicide [4], fungicide [5,6], larvicide [7,8], insecticide [9], or leishmanicidal [10,11,12] with application in different sectors, have been demonstrated for certain essential oils. In addition a large number of researches are conducted about the antioxidant activity of the essential oils [13,14,15], because oxidation process is responsible of the biological substances damages and subsequently cause of many disorders, including atherosclerosis, arthritis, diabetes, cancer, Parkinson's disease or Alzheimer disease [16,17,18,19,20,21,22]. From a chemotaxonomic point of view, the essential oil composition has been particularly helpful in assessing taxonomic relationships [23] of several species with high similarities in morphological characters especially in leaves, trichomes, and flowers. It has been discussed the convenience or not to use the volatile components of plants as taxonomic criteria, due to the high variability observed in different populations of the same species or even between species of the same population [24]. However if the essential oil composition, taken separately may be lower than other phytochemical groups such as flavonoids, diterpenes or alkaloids, this inferiority can be offset by using the concentration of different main compounds in the plant or better yet, the presence or absence of several components because it reflect the outcome of a series of metabolic processes that although affected by environmental variables (soil, climate) or age of the aromatic plant must be the results of its biochemistry and genetic structure [25]. In this sense, the essential oil composition has been of great help in solving chemotaxonomic problems in several genera and species of different families like Labiatae, i.e. *Salvia* [26], *Teucrium* [27,28], *Satureja* [29] or *Thymus* [30]. The aim of this overview is to summarize some aspects of the essential oil composition, to emphasize about the herbicidal activity in order to use the essential oils in sustainable agriculture and to focus the antimicrobial activity exhibited by some essential oil in controlling bacterial virulence factors such as biofilm formation or in the use as natural antimicrobial agents in edible packaging materials.

## 2. ESSENTIAL OIL COMPOSITION

The wide range of biological activities showed by the essential oil is related to the qualitative and quantitative composition of these natural volatile mixtures. We can found terpenoids (monoterpenes, sesquiterpenes and diterpenes in the form of hydrocarbons, alcohols, aldehydes, ketones, ethers, esters, peroxides and phenols), aromatic compounds (C<sub>6</sub>-C<sub>3</sub> and C<sub>6</sub>-C<sub>1</sub> compounds) less frequent but characteristic of certain essential oils and low

molecular weight aliphatic compounds (hydrocarbons, alcohols, acids, aldehydes, esters and lactones) with different physical, chemical and pharmacological properties, responsible of the activity of whole essential oil. It is very important to know not only botanical classification but also their origin, harvest time, extraction process and analytical techniques in order to ensure quality in both chemotaxonomic and pharmacological purposes. To obtain essential oils from aromatic plants only the conventional method, steam distillation (hydrodistillation, saturated steam, dry steam) must be employed according to the recommendations of the European Pharmacopoeia [31].

Innovative techniques, ultrasound assisted extraction and microwave assisted extraction techniques such as in situ microwave-generated hydrodistillation, microwave steam distillation, microwave hydrodiffusion and gravity, and microwave steam diffusion than can reduce the time of extraction may be used [32], but could be modified their natural composition, mainly the quantitative composition. It is well known that the essential oil of several species are different according to the geographical origin, and in this sense for *Artemisia arborescens*, chamazulene (American oil) or  $\beta$ -thujone (Morocco oil) type has been reported [33]. The quantitative prevalence of  $\beta$ -thujone ( $33.4 \pm 4.55$ ;  $42.6 \pm 6.13$ ) and chamazulene ( $28.1 \pm 3.64$ ;  $24.4 \pm 6.56$ ) at the vegetative and flowering stage respectively was the prominent chemical characteristic of the *A. arborescens* essential oil from Sicilian [34]. In other cases geographical origin affects both qualitative and quantitative composition of the essential oils. In this way *Lantana camara* L., an aromatic shrub native to tropical America, considered one of the worst weed [35], grows in Europe and other regions as ornamental plant. Although their essential oil contains the sesquiterpene hydrocarbons as the main phytochemical group, there are many differences with other fractions and also with the main compounds. In Spain monoterpene fraction, both oxygenated and hydrocarbons were not detected being the main compounds the sesquiterpene hydrocarbons  $\alpha$ -curcumene (23.1%) followed by  $\beta$ -caryophyllene (17.5%) and  $\gamma$ -curcumene (14.6%) [36] whereas in Algeria and India *L. camara* elaborate an essential oil with monoterpene compounds and with great differences in the sesquiterpene oxygenated fraction.  $\beta$ -caryophyllene was the main compound in both countries (35.7% and 23.3% respectively), followed in Algeria by caryophyllene oxide (10.0%) [37] whereas this oxygenated sesquiterpene only reach 0.3 % in India [38] being the main compound of this fraction davanone (7.3%), compound which was only present in 0.3% in the essential oil of *L. camara* from Algeria. Together with the geographic origin of the specie it is highly relevant to know the variety. Certain bioactive compounds such as zerumbone (Fig. 1) with interesting antitumor [39,40] and antiinflammatory [41,42] properties only occur in the essential oil of several *Zingiber* species and varieties. Zerumbone was not present in the essential oils obtained by hydrodistillation in a Cleveger type apparatus from the rhizome of *Z. officinale* (L.) Roscoe in samples collected in India ( $\alpha$ -zingiberene 8.2%, geranial 15.0% and neral 10.6%) [43] and Brasil ( $\alpha$ -zingiberene 23.8%, geranial 14.2% and (E,E)- $\alpha$ -farnesene 10.0%) [5], being found in large amount in the essential oils also obtained by hydrodistillation from the fresh rhizomes of *Zingiber zerumbet* Smith (zerumbone 88.5%,  $\alpha$ -humulene 2.3%) [43] and *Zingiber zerumbet* var. *darcy* (zerumbone 69.9%,  $\alpha$ -humulene 12.9%) [44] collected in India (Fig. 1). Both essential oils became the best natural biological source for the isolation of the oxygenated sesquiterpene zerumbone used in pharmacological assays.



**Fig. 1. Gas chromatography chromatogram to *Z. zerumbet* (L.) Smith essential oil and mass spectral of zerumbone (peak 40.80 min)**

Finally, regarding the chemical composition of an essential oil, GC-FID (Gas Chromatography coupled with Flame Ionization Detection) and GC-MS (Gas Chromatography - Mass spectrometry) are the best standard techniques for their analysis being employed for more than 90% of the researchers. Although other associated spectroscopic techniques, including IR (Infrared), FT-MIR (Fourier Transform-Middle Infrared spectrometry) Nuclear Magnetic Resonance ( $^1\text{H}$  and  $^{13}\text{C}$ -NMR), HPLC-UV (High Performance Liquid Chromatography coupled with Ultra-Violet detection) HPLC-Mass (High Performance Liquid Chromatography - Mass Spectrometry) could be advantageous in the

identification and quantification of certain compounds in several essential oils [45,46] are more expensive analysis. In summary, standardized processes with these volatile constituents are essential to assure a constant biological activity.

### 3. BIOLOGICAL ACTIVITIES OF THE ESSENTIAL OILS

Essential oils in addition to their aromatic properties own biological activities closely linked to their chemical composition and related with environment and human health. Their use to treat respiratory tract, digestive system or skin infections diseases have been confirmed. Patent US 7048953 [47] describes methods of inhaling the vapors of essential oils, including eucalyptus oil (*Eucalyptus globulus*) and tea tree oil (*Melaleuca alternifolia*) to prevent, treat, and cure infections of Severe Acute Respiratory Syndrome (SARS) and more recent patents are about essential oils or their components in new promising biological activities such as herbicidal or inhibition of both microorganism growth and biofilm formation [48,49].

#### 3.1 Herbicidal Activity

The development of agrochemical products between 1940-1990s, based on the employment of chemical fertilizers and pesticides increased agricultural productivity. However the overuse of agrochemicals such as synthetic herbicides involved the emergence of resistant strains of weed and also its accumulation in soils and ground water with adverse effects in living organism and human health. The firsts resistances were observed in the 50s, in flies and mosquitoes, but the real health problem was demonstrated when it was found that not only was produced in insect of agricultural plagues, but also in other transmitters of human diseases, such as the emergence of resistance in the vectors of malaria in 1960 [50]. This has driven the search of alternative methods for weed control, based on natural products possessing safety with both environmental and human health. Between natural substances described as inhibitors of seed germination and seedling growth we can found cinnamic acid derivatives, coumarins, flavonoids, alkaloids, cyanoglycosides and amino acids together terpenoids, including volatile terpenes that are the main compounds of the essential oils [51,52,53,54,55,56,57,58,59]. In plants, it is well known that essential oil plays an important role in defense mechanism, especially against herbivores, damaging insects or fungal pathogens [60]. Volatile terpenoids also act in plant-plant interactions, being attractant agents in insect pollination [61]. Several essential oils or its components have been employed in different herbicide formulations [48,62]. The advantage of using volatile herbicides is due to their low persistence in the field when compared with nonvolatile herbicides [63], being their use promising in organic agriculture for weed control, but also the main problem is its volatility needing to emulsify the essential oil with surfactants to improve efficiency or using alternative formulations, such as microencapsulation that are recently develop to increase the duration its effect, reducing volatilization and slowing its degradation in the environments [64,65,66]. Several essential oil show great species-specific toxicity against seed germination, without effects or even with stimulatory effect in others [36,67]. Preliminary studies showed than oxygenated monoterpenes are more phytotoxic compounds than monoterpene hydrocarbons [68,69,70,71]. In this way carvacrol, carvone, thymol, *trans*-anethole and linalool were the most phytotoxic components completely inhibiting rigid ryegrass germination and root length at 160 nL/cm<sup>3</sup>, whereas the monoterpene hydrocarbons myrcene,  $\beta$ -pinene,  $\alpha$ -pinene, limonene, ocimene and *p*-cymene were slightly phytotoxic [72]. Also herbicidal activity was showed when a mixture of oxygenated monoterpenes (carveol, carvone, menthone, and carvyl acetate) together the oxygenated sesquiterpene cedrol was tested in green house on paddy weeds [73,74], being the high

content of the oxygenated monoterpenes nepetalactones (92.23%), in *Nepeta meyeri* Benth essential oil, the responsible of the inhibition of seed germination and seedling growth of *Amaranthus retroflexus* L., *Portulaca olerace* L., *Bromus danthoniae* Trin., *Agropyron cristatum* L., *Lactuca serriola* L., *Bromus tectorum* L., *Bromus intermedius* Cuss., *Chenopodium album* L., *Cynodon dactylon* L. and *Convolvulus arvensis* L. [75].

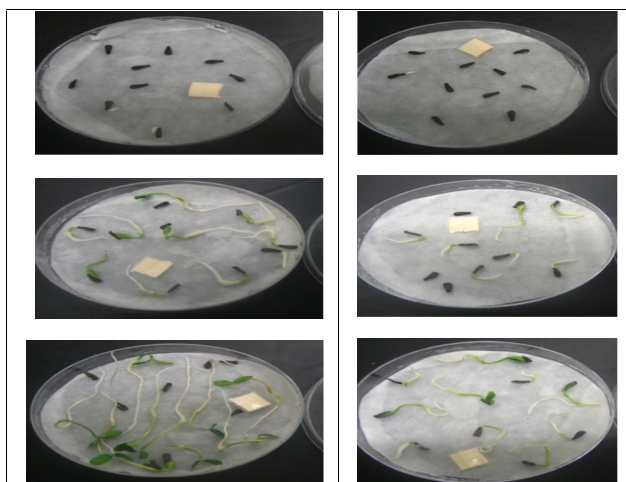
Herbicidal activity was also observed with oxygenated monoterpene derivatives, so hydroxy and ester derivatives of 1,8-cineole (main compound in *Eucalyptus* sp. essential oils) and 1,4-cineole showed a dose-dependent herbicidal activity against annual ryegrass (*Lolium rigidum*) and radish (*Raphanus sativus* var. long Scarlet) [76]. Others oxygenated monoterpenes such as camphor, 1,8-cineole, borneol, thymol and carvacrol are responsible of inhibitory effect in both crops and weeds [51,77,78,79]. In this sense the high content of artemisia ketone (56.46%) in the essential oil of *Eriocephalus africanus* L. [36] can be explain their phytotoxic activity against Mediterranean summer crops, as occur in other *Artemisia* species with allelopathic properties [80]. However not only monoterpene compounds are responsible for germination inhibition [70,71], being the strong inhibitory effect of *Eucalyptus camaldulensis* Dehn. essential oil [36] attributed to the large content of oxygenated sesquiterpenes (48.27%) mainly by the presence of spathulenol (41.46%) suggesting according to other authors [77] than 1,8-cineole is not the principal responsible for the phytotoxic effect of *Eucalyptus* sp. essential oil.

In other cases the essential oil is more active than their main oxygenated monoterpene compound. Clove essential oil (*Syzygium aromaticum* (L.) Merr. and Perr. caused greater inhibition of seedling growth against broccoli (*Brassica oleracea* var. *italica*), common lambsquarters (*Chenopodium album* L.) and redroot pigweed (*Amaranthus retroflexus* L.) than their main compound eugenol [81]. Or even essential oil with high content on monoterpene hydrocarbons can be more effective than essential oil with large amount of oxygenated monoterpenes against certain weed. A comparative study between leaves essential oils of *Citrus limon* (L.) Burm.f., *Citrus sinensis* (L.) Osberk, *Myrthus comunis* L. and *Laurus nobilis* L., all rich in monoterpene fractions against the same weed (*Araujia sericifera* Brot.) showed that the more phytotoxic effect depended of the monoterpene hydrocarbons. The most effective essential oils against seed germinations was lemon essential oil, completely inhibiting seed germinations at 0,250,0,5 and 1  $\mu\text{L}/\text{mL}$  (unpublished data), followed of orange essential oil, myrtle essential oil and bay leaf essential oil (Fig. 2). *C. sinensis* essential oil with 69.72% of monoterpene hydrocarbons was more active than *Myrthus comunis* L. and *Laurus nobilis* essential oils with 74.76% and 60.82% respectively of the oxygenated monoterpenes. The main compounds of *C. sinensis* was sabinene  $30.09 \pm 2.88$ , *trans*-ocimene  $9.05 \pm 0.66$  and  $\delta$ -3 carene  $8.50 \pm 2.06$ , however the less active essential oil *L. nobilis*, contains large amounts of oxygenated monoterpenes 1,8-cineole  $33.13 \pm 7.37$ ,  $\alpha$ -terpinyl acetate  $13.02 \pm 2.25$  and the phenylpropanoid methyl eugenol  $12,88 \pm 1,79$  (unpublished data). It is interesting to note that the most effective essential oils against *A. sericifera*, a weed in *Citrus* is accurately the essential oils produced in the leaves of cultivar *Citrus* trees from Valencia (Spain).

On the other hand, when we compare *in vitro* the phytotoxic activity between the essential oils and aqueous extracts of the same biological raw material, in general *in vitro* essential oils are more active in both seed germination and seedling growth against weeds, but again it is related to the composition and weed applied [36,82]. The essential oil of *E. camaldulensis* Dehn. was the most effective, completely inhibiting both *Amaranthus hybridus* L. and *Portulaca oleracea* L. seed germination [36], and also *E. camaldulensis* aqueous extract was the most effective reducing *A. hybridus* germination. *Eriocephalus*

*africanus* L. extract exhibited significant activity up to 30% concentration, while *Lantana camara* L. extract was least active. Against *P. oleracea*, *E. africanus* extract obtained the best results followed by *E. camadulensis* extract, while *L. camara* extract showed no inhibitory effect [82]. In greenhouse assays (in trays with soil), the essential oils were less effective whereas all aqueous extract showed herbicidal activity against the same weed. *E. africanus* extract was the most effective (52.45% inhibition), followed by *E. camaldulensis* (38.43%) controlling seed germination during 6 week, whereas *L. camara* was less active (21.32%). In field conditions, *E. africanum* and *E. camaldulensis* extracts corroborated the results obtained under greenhouse conditions (68.9 and 39.6% maximum inhibition respectively); however *L. camara* was no active showing even stimulatory effect [83]. Although the essential oil of *Ocimum basilicum* L. against tomato, lettuce and melissa corroborated that the essential oil is more active than aqueous extract [84] in other species such as *Tagetes minuta*, the phytotoxic effect of aqueous extract is higher than their essential oil [85].

Accordingly, the herbicidal potential of the essential oils depends on the composition, doses employed as well as on the weed against they are applied, being possible to develop specific herbicides for a particular weed.



**Fig. 2. Control (*A. sericifera*) and treatment with essential oil of *Laurus nobilis* after 5, 10, 14 days of incubations (unpublished data)**

### 3.2 Antimicrobial Activity

Essential oils possess antimicrobial activity [86] and have been used for longtime [87,88,89] in folk medicine to treat infections, even replacing antibiotics in mild infectious diseases.

The existence of resistance development for commonly used antibiotics increase the search of natural additives with antimicrobial activity. The selective toxicity towards several pathogens and the synergism of individual components with different mechanism of action than hardly can develop resistance is the main remarkable properties to the essential oils. In food industry this antibacterial activity has been focused in both, directed against many food pathogens and food spoiling bacteria, including the biofilm formation inhibition making these

substances attractive as food preservatives and in the use of natural antimicrobial agents in edible packaging materials.

### **3.2.1 Food antimicrobial agents. Biofilm formation inhibitors**

Antimicrobial activity of the essential oils is highly related with their qualitative and quantitative composition. L'Aromatogramme of thyme essential oils [90] showed that the oxygenated compounds play an important role in the antimicrobial activity against *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 18883, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Candida albicans* ATCC 26555 and *Mycobacterium phlei* CECT 3009. Although *S. aureus*, *C. albicans* and *M. phlei* were inhibited by the same strip of *Thymus leptophyllus* and *T. webbianus* essential oils, the essential oil of *T. webbianus* showed a specific band against *C. albicans* attributed to the sesquiterpene hydrocarbons germacrene B (18.8%), calamenene,  $\gamma$  and  $\delta$ -cadinene found in this oil.

Phenolic compounds commonly found in essential oils (thymol, carvacrol and eugenol) have antimicrobial activity against foodborne pathogens [91,92]. In agar well diffusion method thymol, carvacrol as well as thyme essential oil showed inhibitory effect on *Shigella sonnei* and *S. flexneri*, whereas eugenol by disc-diffusion method, at 0.0125% MIC (minimal inhibitory concentration,) and 0.025% MBC (minimum bactericidal concentration) produced complete inhibition of *Salmonella typhi*. The antibacterial activity of eugenol against *Salmonella typhi* according to the authors is due to the interaction on bacterial cell membrane [92]. The damage in membrane integrity was also observed in the total inhibition of *Pseudomonas aeruginosa* and *Staphylococcus aureus* with thymol and carvacrol [93]. In addition to phenolic compounds, oxygenated monoterpenes (linalool, 1,8-cineole,  $\alpha$ -terpineol) or even monoterpene hydrocarbons ( $\alpha$ -pinene) compounds usually presents in many essential oil are responsible of the antimicrobial activity against potential food spoilage microorganisms [94]. The most effective (bacteriostatic/bactericidal effects) antimicrobial natural compound against *Aeromonas hydrophila*, *Escherichia coli*, *Brochothrix thermosphacta*, and *Pseudomonas fragi*, was thymol (40 pp/100ppm) followed by carvacrol (50 ppm/100 ppm), linalool (180 ppm/720 ppm),  $\alpha$ -pinene (400 ppm/no bactericidal effect), 1,8-cineol (1,400 ppm/2,800 ppm), and  $\alpha$ -terpineol (600 ppm/no bactericidal effect). Thymol and carvacrol were the most effective combination against all tested bacteria. The addition of linalool to each showed a synergistic antibacterial effect depending on their concentration. Phenolic compounds combined with  $\alpha$ -terpineol led to a synergistic antimicrobial effect against *A. hydrophila*, *E. coli*, and *P. fragi* however produced antagonistic effects against *B. thermosphacta* [94]. Regarding essential oils, *Laurus nobilis* essential oil (1,8-cineole 39.81%) showed bacteriostatic and bactericidal effects against *Salmonella enteric* subsp. *enterica* serovar Senftenberg (CECT 4563), *Escherichia coli* O157:H7,22, *Escherichia coli* (CECT 471), *Yersinia enterocolitica* (CECT 4315), *Staphylococcus aureus* (CECT 976), *Enterococcus faecium* (CECT 4932), *Listeria monocytogenes* (CECT 4031), *Listeria monocytogenes* EGD-e and *Bacillus subtilis* (CECT 4071) whereas *Myrtus comunis* essential oil with large amount of myrtenyl acetate (49.27%), 1,8-cineole (26.93%) and  $\alpha$ -pinene (16.52%) was not found to be bactericidal activity [95].

Although, many essential oils compounds possess antibacterial properties, even higher than oleoresin their strong flavoring properties limit their usage as food antimicrobial agents [96]. Search of essential oils with potent bactericidal effect and do not affect the flavor and taste



of food products is the first limitation of use. Food quality and safety is essential for both consumers and food industry.

Antimicrobial activity of essential oils can be reduced by storage conditions and also by bacteria (e.g. *Pseudomonas* sp., *Staphylococcus aureus*) with the ability to produce biofilms with an additional protection against a wide range of antimicrobials [97,98]. Biofilms have been implicated in serious human infections and are responsible of 90% of chronic infections. Bacterial biofilms are complex communities of bacteria embedded in a self-produced matrix and attached to inert or living surfaces. These microorganism communities are more resistant to both antibiotics and immune system. Microbial biofilms are an important problem in food and pharmaceutical industries, being responsible of principal foodborne diseases. In the United States yearly, 31 pathogens caused 37.2 million (90% CrI 28.4-47.6 million) illnesses, of which 36.4 million (90% CrI 27.7-46.7 million) were domestically acquired; of these, 9.4 million (90% CrI 6.6–12.7 million) were foodborne. The 59% of foodborne illnesses were caused by viruses, 39% by bacteria and 2% by parasites [99].

Natural compounds controlling bacterial virulence factors such as biofilm formation (that show also high resistance against cleaning and disinfecting reagents) are interesting to prevent the development of resistant strains [100].

Spices in food products are mainly used to enhance flavour and taste, but also have antimicrobial properties [101] and in this sense the essential oils of spices, *Citrus* [102] or other genera could be applied as natural food preservatives [103].

Several studies are aimed in order to evaluate if essential oils with demonstrated antimicrobial activity are able to inhibit biofilm formation and therefore have effect to prevent foodborne diseases. Oregano essential oil, carvacrol and thymol are able to reduce biofilm formation in several strains of *Staphylococcus aureus* and *S. epidermidis* [104]. Similar results were found with thyme essential oil, oregano essential oil and carvacrol against *Salmonella typhimurium* [105]. More recently Szczepanski and Lipski [106] reported that thyme, oregano and cinnamon essential oils showed inhibiting effect on biofilm formation below their minimal inhibitory concentration. The three essential oils showed antimicrobial effect on planktonic cell of strains of the genera *Acinetobacter*, *Sphingomonas* and *Stenotrophomonas*. The minimal inhibitory concentration (MIC) of the essential oils ranged from 0.016% for thyme and oregano essential oils on *Sphingomonas* spec. up to 0.063% for cinnamon essential oil on *Acinetobacter* spec. [106]. The isolate of *Stenotrophomonas* genus showed highest MIC against all tested essential oils, being not effective in biofilm formation. Biofilm formation of the *Sphingomonas* and the *Acinetobacter* strain was inhibited below 50% of the minimal inhibitory concentration of the essential oil, whereas the biofilm formation of the *Stenotrophomonas* strain was not inhibited below MIC. On the other hand, mandarin essential oil with high content of monoterpene hydrocarbons limonene (89.82%),  $\gamma$ -terpinene (2.76%) and myrcene (2.51%) was not active against *Pseudomonas aeruginosa* ATCC 27853 growth, however at the same doses (1, 0.1 mg/ml) the inhibition of bacterial biofilm formation was similar and higher respectively to azithromycin at 5 $\mu$ g/ml used as positive control [98]. So, essential oils with high content in both oxygenated and hydrocarbons monoterpene can inhibit biofilm formation from specific foodborne bacteria.

### **3.2.2 Food antimicrobial agents. Edible package antimicrobial**

Reduce or eliminate food-related microorganisms without negative effects on food quality throughout the shelf life of food products is a challenge to food industry. The use of antimicrobial biodegradable films (a partial alternative to plastic packaging) are increasing in order to delay microbial spoilage of food and reduce or inhibit foodborne pathogens.

In concerning to the incorporations of essential oils into edible films it is important to know the effect that they can produce [107,108]. In some cases, such as occur with the addition of oregano essential oil to alginate films, the obtained films were less rigid, more flexible, and less transparent, compared with the control film [109].

Among the many materials, proteins, lipids and polysaccharides that can form edible films, alginate a water-soluble polysaccharide and chitosan [109,110,111] has been extensively investigated.

The essential oils of clove (*Syzygium aromaticum* L.), cumin (*Cuminum cyminum* L.), caraway (*Bunium persicum* Bioss.), marjoram (*Origanum majorana* L.), cinnamon (*Cinnamomum zeylanicum* Blumen), coriander (*Coriandrum sativum* L.), fennel (*Foeniculum vulgare* Miller), cypress (*Cupressus sempervirens* L.), lavender (*Lavandula angustifolia* Miller), thyme (*Thymus vulgaris* L.), herb-of-the-cross (*Verbena officinalis* L.), pine (*Pinus sylvestris* L.) and rosemary (*Rosmarinus officinalis* L.) have been tested against common foodborne pathogens in order to determine their antimicrobial activity (*Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas fluorescens*, *Shewanella putrefaciens*, *Photobacterium phosphoreum*, *Listeria innocua*, *Lactobacillus acidophilus*, *Pseudomonas putida*, *Streptococcus agalactiae* or *Lactococcus lactis*) before and after their incorporation into several films [112,113,114]. The antimicrobial activity varies according both the essential oil used and microorganism tested. Between the essential oils tested against *S. aureus*, *E. coli* and *L. monocytogenes*, marjoram, essential oil showed the stronger antibacterial activity followed to clove, cinnamon, coriander, caraway and cumin [113]. Clove, thyme and rosemary were the most active against *Lactobacillus acidophilus*, *Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas fluorescens*, *Photobacterium phosphoreum* and *Shewanella putrefaciens* [112]. In general, gram-negative microorganisms are more resistant due to the external lipopolysaccharide wall which may prevent active components from reaching the cytoplasmic membrane [103].

When essential of marjoram, clove and cinnamon were incorporated to alginate-clay nanocomposite film, regardless of the composition of essential oil (terpinen-4-ol, eugenol, and cinnamaldehyde respectively as the main compound), the antimicrobial activity was maintained, but essential oil-enriched film showed less antibacterial activity in comparison with pure essential oil [113]. Similar results were obtained when oregano essential oil incorporated into alginate films was tested against *Staphylococcus aureus*, *Listeria monocytogenes* and Gram-negative bacteria as *Escherichia coli* and *Salmonella enteritidis* [109]. In general the activity of food packaging films depends on the nature and amounts of essential oil as well as the microorganisms tested and the effectiveness of the films tends to decrease during the storage period [113].

It is possible also develop antimicrobial films by the combinations of both an essential oil with mixture of films such as gelatin-chitosan biodegradable films [112] or two or three essential oils in the film matrix [115]. No differences between matrices (bovine-hide gelatin and chitosan edible films) in the antimicrobial activity were observed when clove essential oil

was incorporated. However some differences occur in water solubility between gelatin-chitosan-clove film and gelatin-clove film. The lower solubility of gelatin-chitosan-clove may be advantageous because essential oil could be released more slowly and maintain their effect for longer [112].

Chitosan enriched by lemon, thyme and cinnamon essential oil was investigated against *E. coli* and *S. aureus*. Chitosan-thyme essential oil exhibited the best antibacterial effect followed by the cinnamon and lemon essential oils. The combined use of two essential oils not caused synergistic effects being their antibacterial properties similar to a single essential oil, however the combination of two essential oils in the chitosan film had better water barrier properties and lower particle size [115]. This results shows that single essential oil or mixtures of them can be employed to enhance the antimicrobial activity against a particular microorganism.

#### 4. CONCLUSION

The herbicidal and antimicrobial activities of essential oils is correlated with their chemical composition and depend of the doses employed as well as the weed or microorganisms against they are applied, being possible to develop specific herbicides or preservative substances for a particular weed or pathogen.

The use of essential oils providing additional antimicrobial (and/or antioxidant) property to edible films and coating, taking into account other aspects such as color or opacity of the films that must be accepted by the consumers, can extend shelf-life of food and become in an attractive option against foodborne diseases.

#### COMPETING INTERESTS

Author has declared that no competing interests exist.

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