Antibacterial Properties of *Cymbopogon martinii* Essential Oil against *Bacillus subtilis* Food Industry Pathogen [†]

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Abstract: Essential oils have great potential in the field of the food industry as they can effectively prevent the presence of several bacterial and fungal pathogens. Essential oils are complex volatile compounds, synthesized naturally in different plant parts during the process of the secondary metabolism. The main goal of this work is to perform a qualitative evaluation of the antibacterial properties of 24 chemotyped essential oils against the growth of *Bacillus subtilis*. These Gram-positive bacteria are responsible for “rope” disease in bread preservation processes. The study was carried out using the method of disk-diffusion in agar. Biological activity was observed in five essential oils: *Cymbopogon martinii* var. *motia*, *Thymus vulgaris* QT Linanol, *Thymus satureioides*, *Mentha piperita* and *Eugenia caryophyllus*. The first three have in common the presence of some mono terpenic derivatives—Geraniol, Linalool and Carvacrol, respectively—with strong antimicrobial effects. The *Cymbopogon martinii* essential oil is one of the botanicals with the highest geraniol content (up to 80.53%) and showed more activity antimicrobial than the others. A contributing role of this knowledge could be the design of *Cymbopogon martinii* essential oil formula, which can be used in bakery industry as a preservative, such as nano-encapsulation for bakery doughs, active packaging of baked products or surface disinfectants.

Keywords: essential oils; *Bacillus subtilis*; *Cymbopogon martinii*; antibacterial activity; geraniol; bakery industry

1. Introduction

Essential oils (EO) are aromatic and volatile liquids extracted from plants material, such as flowers, aerial parts, roots, bark, leaves and fruits (Burt, 2004). The chemical composition of EOs is complex, one of them, there may have around of 20–60 different bioactive components and only two or three are the major components at concentration within a range of 20–70%; the others are in traces. These composition of EOs from a one species of plant can differ from the geographic location, the harvesting seasons or extraction method [1,2].

EOs are secondary metabolites formed by plants; their main role is to protect them against conditions of biotic and abiotic stress. They constitute about 1% of plant secondary metabolites and are mainly represented by terpenoids, phenylpropanoids or benzenoids, fatty acid derivatives and amino acid derivatives [3].

The mechanisms of antimicrobial action of EOs is mediated by a series of biochemical reactions, it is dependent on the type on chemical constituents [4]. Although the exact mechanisms of bacteriostatic or bactericidal activity and antimicrobial effects of EOs are not exactly known, they are

known to cause structural and functional damage to the membrane of bacteria by various antimicrobial mechanisms or alteration of proton pump [5–9].

The EOs are used in food industry for food preservation, due to aroma, flavors and natural antimicrobial contents against pathogenic bacteria [10–12]. Although the mechanism of action is not known in great detail, it is necessary consider the large number of different groups of chemical compounds present in EOs; it is most likely that their antibacterial activity is not attributable to one specific mechanism, as there are several targets in the cell [13].

Cymbopogon martinii var. *motia*, *Thymus vulgaris* QT *Linanol*, *Thymus satureioides*, *Mentha piperita* and *Eugenia caryophyllus* are used traditionally as food additives [14–16]. These properties have been attributed to EOs contained in the species, as well as the presence of non-volatile compounds including polyphenols and flavonoid [11]. The aim of this study was to evaluate the effect of essential oils on growth, spore production of *Bacillus subtilis* that could alternative synthetic chemical preservatives in bakery industry.

2. Materials and Methods

2.1. Bacterial Strains and Media

The *Bacillus subtilis* (Ehrenberg 1835) Cohn 1872, strain CECT 4522 was used in this study. *B. subtilis* was maintained on nutrient broth medium and solidified. Growth temperature was 30 °C and the incubation time was 48 h. Nutrient broth medium (Beef extract, 0.5%; Peptone, 1%; NaCl, 0.5%) was adjusted to pH 7.2.

2.2. Essential Oils (EO)

Essential oils, chemotyped, are extracted from different plants by steam distillation (Pranarôm, S.A., 7822, Ath, HAINAUT Belgium) The twenty four EOs used for the study were: *Citrus sinensis*, *Citrus reticulata*, *Elettaria cardamomum*, *Laurus nobilis*, *Cymbopogon martinii* var. *motia*, *Zingiber officinale*, *Eugenia caryophyllus*, *Cinnamomum camphora*, *Rosmarinus officinalis*, *Melaleuca quinquenervia*, *Chamaemelum nobile*, *Melaleuca alternifolia*, *Thymus vulgaris* CT LINALOL, *Citrus paradisi*, *Citrus junos*, *Origanum compactum*, *Mentha x piperita*, *Myrtus communis*, *Curcuma longa*, *Cinnamomum cassia*, *Thymus satureioides*, *Eucalyptus radiata* ssp *radiata*, *Cinnamosma fragrans* and *Mentha arvensis*.

2.3. Antibacterial Activities of Essential Oils

The assessment of the antibacterial activities of essential oils was performed by the diffusion method. The disc absorption capacity was 5 µL/disc; only the disk diffusion assay with EOs was conducted to detect antimicrobial activity. Sterile disks were impregnated with 5 µL of EO at different concentrations by serially diluted in vegetal oil (100%, 10%, 1%) (v/v) and each disk was placed on a nutrient broth agar plate smeared with *B. subtilis*. The plates were incubated for 48 h at 30 °C to determine the antimicrobial effect. Antibacterial activity was determined by measuring the inhibition zone diameter (mm) against each EOs (Table 1). Each report was realized in two different experiments. A sterile vegetal oil without EO was used as a negative control.

3. Results and Discussion

EOs and their constituents play a key role in exerting antimicrobial activity; the results of screening of twenty-four EOs (Table A1) were evaluated against *B. subtilis*.

The inhibition of EOs was shown as an inhibition diameter against the bacterial growth (Table 1), while the control with vegetal oil does not affect the growth of bacteria. When we compared the inhibition results at different dilutions, most of the EOs used without any dilution of the commercial EO showed a zone of inhibition. In 21 of the 24 oils tested, it was observed that the inhibition zone was reduced at less oil concentration; thus, dose-dependent response was clear for each essential oil. The citrus EOs used showed no activity against *B. subtilis*, except for *Citrus junos*, which showed activity.

We are going to focus our attention on the five oils that showed the highest antimicrobial activity at higher concentrations: *Cymbopogon martinii* var. motia (palmarosa oil), *Thymus vulgaris* QT Linanol (thymus oil), *Thymus satureioides* (Moroccan thyme oil), *Mentha piperita* (peppermint oil) and *Eugenia caryophyllus* (clove oil).

Cymbopogon martinii var. motia exhibited the most potent antibacterial activity among all the essential oils tested. However, although *Mentha x piperita* and *Thymus satureioides* had more moderate activity, they were more effective at lower doses.

Eugenia caryophyllus and *Thymus vulgaris* CT LINALOL, showed no activity at higher dilution (100%), similar to *Cymbopogon martinii* var. motia. However, unlike the latter EO, the former EOs were less effective.

Table 1. Antibacterial activity of essential oils.

Essential Oil	Inhibition Zone of EO Concentration (in mm)		
	100%	10%	0.1%
<i>Origanum compactum</i>	2 mm	0. mm	0 mm
<i>Cymbopogon martinii</i> var.motia	8 mm	0.5 mm	0 mm
<i>Eugenia caryophyllus</i>	3 mm	0.2 mm	0 mm
<i>Mentha arvensis</i>	3 mm	0.2 mm	0 mm
<i>Mentha x piperita</i>	4 mm	0.6 mm	0.2 mm
<i>Thymus vulgaris</i> CT Linanol	4 mm	1 mm	0 mm
<i>Thymus satureioides</i>	3 mm	0.2 mm	0.2 mm
<i>Chamaemelum nobile</i>	2 mm	0 mm	0 mm
<i>Citrus sinensis</i>	0 mm	0 mm	0 mm
<i>Citrus reticula</i>	0 mm	0 mm	0 mm
<i>Elettaria cardamomum</i>	2 mm	0.5 mm	0.2 mm
<i>Laurus nobilis</i>	1 mm	0.2 mm	0 mm
<i>Zingiber officinale</i>	0.5 mm	0 mm	0 mm
<i>Cinnamomum camphora</i>	1 mm	0.2 mm	0 mm
<i>Rosmarinus officinalis</i>	0.5 mm	0 mm	0 mm
<i>Melaleuca quinquenervia</i>	1 mm	0.5 mm	0 mm
<i>Melaleuca alternofolia</i>	0.5 mm	0.2 mm	0 mm
<i>Citrus paradisi</i>	0 mm	0 mm	0 mm
<i>Citrus junos</i>	1 mm	0.5 mm	0 mm
<i>Myrtus communis</i>	0.5 mm	0 mm	0 mm
<i>Curcuma longa</i>	1 mm	0.5 mm	0.2 mm
<i>Cinnamomum cassia</i>	0.5 mm	0.2 mm	0 mm
<i>Eucalyptus radiata</i> ssp radiata	2 mm	0.8 mm	0 mm
<i>Cinnamosma fragrans</i>	0.5 mm	0 mm	0 mm

The biological activity of EOs depend of composition of volatile principles such as terpernes, terpenoids, phenol-derived aromatic components and aliphatic components. They represent a natural source of bioactive compounds. In Table A1, we can see some of the terpenic derivatives and phenylpropanoids with more representatives are present in the EOs analyzed. Carvacol and thymol is present in *Thymus satureioides*, eugenol is found in *Eugenia caryophyllus*, geraniol and linalool is present in *Cymbopogon martinii* var.motia, the limonene in *Mentha x piperita* and linalol in *Thymus vulgaris* CT Linanol. Other authors have also shown the antibacterial activity of these monoterpenes to be present in EO and their use in food industry [17,18]. However, it is important to consider the synergism potential of several volatile components in this effect antimicrobial [10]. The synergistic of EOs with various nanocarriers plays an emerging role in the food industry [19]. Development of techniques such as microencapsulation has the ability to enhance the oxidative stability, thermostability, shelf-life and biological activity of oils [20].

Recent studies reveal the antibacterial activity of different EOs applied in bakery products including thyme, cinnamon, oregano and lemongrass, that can inhibit the growth of harmful microorganisms, resulting in a product with extended shelf-life and enhanced safety [21]. Essential oil extracted from *Cymbopogon martinii* showed the highest activity against both Gram-positive and Gram-negative bacteria among the tested essential oils [22]. *B. subtilis*, a Gram-positive bacterium, is responsible for “rope” disease in bread preservation processes [23,24]. The use of palmarosa oil could be considered to avoid this presence of rope in bread.

At the same time, the palmarosa essential oil as an antioxidant in food plays an important role as a health protecting factor. It is highly reactive against free radicals and oxygen species from a wide variety of sources in biological systems [25].

4. Conclusions

The results obtained in this study confirm the antibacterial and antioxidant activities of five essential oils: *Cymbopogon martinii* var. motia, *Thymus vulgaris* QT Linanol, *Thymus satureioides*, *Mentha piperita* and *Eugenia caryophyllus*. All of them contain compounds with antioxidant activity (phenolic compounds) that may be used to prevent the growth of bacteria by damaging their membrane.

In food industry, products must be supplied without any microbial contamination. The possible use of EOs to increase the shelf life and safety of bakery products raises new technological solutions; however, some limitations, such as altered sensory parameters, may limit its application.

The development of techniques such as nano-encapsulation for bakery doughs, active packaging of baked products or surface disinfectants is required. There is present the choice to introduce the *Cymbopogon martinii* essential oil formula in the bakery industry. However, further research is needed to evaluate the safety and effectiveness of this EO in bakery doughs.

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Appendix A

Table A1. Chemical components from the essential oils. The table shows the essential oils analyzed: part of the plant and a percentage of some chemical components of selected essential oil after was subjected to steam distillation (data obtained from analysis sheet of Pranarôm).

Essential Oil	Part Subjected to Steam Distillation	Chemical Components of Selected Essential Oil Constituents (%)
<i>Origanum compactum</i>	Flowery peak.	Carvacrol (57.6%); Thymol (8.21%); γ -Terpineno (14.1%)
<i>Cymbopogon martinii</i> var. motia	Aerial part.	Geraniol (80.5%); Geranyl acetate (8.95%); linalool (2.45%); β -caryophyllene (1.87%)
<i>Eugenia caryophyllus</i>	Flower bud.	Eugenol (79.9%); Eugenyl acetate (12.3%); β -caryophyllene (5.39%)
<i>Mentha arvensis</i>	Aerial part.	Menthol (71.1%); Mentone (5.88%); Isomentone (3.85%); limonene (2.52)
<i>Mentha x piperita</i>	Aerial part.	Menthol (44.5%); Mentone (18.2%); 1,8 cineole (4.64%)
<i>Thymus vulgaris</i> CT Linalol	Flowery peak.	Linalool (68.4%); Linalyl acetate (6.19%); β -myrcene (3.32%)

<i>Thymus satureioides</i>	Flowery peak.	Borneol (33.4%); Thymol (10.6%); Carvacrol (7.85%); β -caryophyllene (5.82%); Methylamine angelate (20.2%);
<i>Chamaemelum nobile</i>	Flower.	Metallyl angelate (15.4%); Hexil isobutyrate (8.31%)
<i>Citrus sinensis</i>	Shell.	Limonene (95.3%)
<i>Citrus reticulata</i>	Shell.	Limonene (71.1%); γ -Terpineno (18.3%)
<i>Elettaria cardamomum</i>	Fruit.	Terpenyl acetate (35.3%); 1,8 Cineole (32.3%); Linalyl acetate (5.35%); Linalool (3.35%)
<i>Laurus nobilis</i>	Leaves.	1,8 cineole (44.9%); Terpenyl acetate (10.5%); Sabinene (8.86%); Linanool (4.43%)
<i>Zingiber officinale</i>	Rhizomes.	α -zingibereno (28.2%); α -Curcumin (7.93%); Camphene (7.9%); β -sesquifelandrene (7.56%)
<i>Cinnamomum camphora</i>	Leaves.	1,8-cineole 56.8%; Sabinene (13.4%); α -terpineol (7.33%)
<i>Rosmarinus officinalis</i>	Flowery peak.	α -Pinene (38.8%); Canfeno (8.88%); Camphor (6.96%); Bornyle acetate (6.94%)
<i>Melaleuca quinquenervia</i>	Flowery peak.	1,8-cineole (50.6%); α -terpineole (8.91%); Limonene (7.48%)
<i>Melaleuca alternifolia</i>	Leave.	Terpinene 4-ol (40.6%); γ -terpinene (21%)
<i>Citrus paradisi</i>	Shell.	Limonene (94.5%)
<i>Citrus junos</i>	Shell.	Limonene (75.6%); γ -terpinene (8.49%); β -felandreno (3.29%)

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