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Original Article

Antimicrobial Effects of Several Essential Oil from Aromatic Plants

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Abstract

Essential oils (EOs) have been long recognized for their antibacterial, antifungal, antiviral, insecticidal and antioxidant properties. The present research aimed to study the antimicrobial effects of some volatile oils from aromatic plants (sweet basil and dill) against several microorganisms, namely *Bacillus subtilis, Alternaria alternata* and *Penicillium expansum*. The oils have been extracted through distillation procedures and the antimicrobial action of the oils was assessed through the disc diffusion method. The best effect against the *Bacillus subtilis* strain has occurred when the essential oil of dill was undiluted. Regarding the the *Alternaria* species, it was noted that dill volatile oil has acted in an efficient way only undiluted. As the oil's concentration decreased, the strain becomed resistant. The sweet basil oil has proven to be highly effective when acting against the *Bacillus* strain. By volatilization, the sweet basil oil produced a strong antimicrobial effect, even in control disc, in which it was noticed a small development of colonies comparing with the dill oil. The results indicated that the sweet basil essential oil exerted an antimicrobial effect both against the tested bacteria and moulds, while the dill oil had a great inhibitory action on *Bacillus subtilis* and *Alternaria alternata*, but was less efficient against *Penicillium expansum*.

Keywords: sweet basil, dill, volatile oil, bacteria, moulds

Introduction

The essential oils (also called volatile oils) are obtained by hydrodistillation of whole tissues or seeds of common culinary herbs, spices and aromatic plants (Jiao *et al*., 2012; Jila *et al.,* 2012; Solorzano Santos *et al.,* 2012; Luu *et al*., 2013; Sandrine *et al.*, 2013; Sibel *et al.,* 2014; Tongnuanchan *et al*., 2014). The volatile oils contain bioactive phytochemicals and may represent natural alternatives to synthetic chemicals used in food preservation (Delaquis *et al.,* 2002). In recent years, a large number of essential oils and their constituents have been investigated for their antimicrobial properties against some bacteria and fungi (Kalemba and Kunicka, 2003).

The influence of essential oils extracted from different aromatic plants on microorganisms has been performed by many authors. Bagamboula *et al.* (2001) studied the effect of basil oil upon two species of *Shigella* found in foods. Burt (2004) analyzed several plant essential oils from setting their antimicrobial effect and the possibility of their use in food preservation. Carmo *et al.* (2008) studied the effect of oregano essential oil on several *Aspergillus* species isolated from foods. Chao *et al.* (2000) investigated the inhibitory effects of 45 oils on 4 Gram positive bacteria and 4 Gram negative bacteria, 2 moulds and one yeast. Daferera *et al.* (2003) tested the effectiveness of seven essential oils (oregano, thyme, dictamnus, marjoram, lavender, rosemary, sage and pennyroyal) on two species of moulds (*Botrytis cinerea, Fusarium solani* var. *coeruleum*) and one bacteria (*Clavibacter michiganensis* subsp. *michiganensis*) with phytopatogen rol. Helander *et al.* (1998) investigated the effect of essential oils compounds (carvacrol, (+)- carvone, thymol, trans-cinnamaldehyde) on two species of the Enterobacteriaceae family (*Escherichia coli O_{157}:H_7* and *Salmonella typhimurium*). Lambert *et al.* (2001) studied the effect of oregano essential oil with carvacrol and thymol on *Staphylococcus aureus* and *Pseudomonas aeruginosa* and noted that certain combination of carvacrol-thymol increased the inhibitory effect of oil of oregano. Lv *et al.* (2011) tested on *Escherichia coli, Staphylococcus aureus, Bacillus subtilis* and *Saccharomyces cerevisiae* essential oil of basil, oregano, bergamot and perilla using agar disk diffusion and broth dilution methods. Analyzing the essential oils by GC/MS, they found that phenols and terpenes were the major antimicrobial compounds in oregano and basil essential oil. The dominant active components of bergamot essential oil were alcohols, esters and terpenes. For perilla essential oil, the major active

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constituents were mainly ketones. Savory, laurel, oregano, basil, cumin, seafennel, myrtle, pickling herb and mint essential oils, combined or single, were tested on several species of microorganisms (*Salmonella typhimurium, Bacillus cereus, Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Yersinia enterocolitica, Saccharomyces cerevisiae, Candida rugosa, Rhizopus oryzae* and *Aspergillus niger*) by Özcan and Erkmen (2001). Rauha *et al.* (2000) investigated 13 phenolic substances and 29 extracts prepared from Finnish plant materials. The microorganisms used in their study were *Aspergillus niger, Bacillus subtilis, Candida albicans, Escherichia coli, Micrococcus luteus, Pseudomonas aeruginosa, Saccharomyces cerevisiae, Staphylococcus aureus* and *Staphylococcus epidermidis*. They found that flavone, quercetin and naringenin were effective in inhibiting the growth of the organisms.

The classical methods commonly used to assessed essential oils antibacterial and antifungal activities are the agar diffusion method (paper disc as well) and the dilution method (agar and liquid both), as well as turbidimetric and impedimetric monitoring of microorganism growth in the presence of tested essential oils (Kalemba and Kunicka, 2003).

Many authors have studied the effect of dill extracts and dill oil on microorganisms. Jirovetz *et al.* (2003) reported a high activity of the essential *A. graveolens* oil against the mold *Aspergillus niger* and the yeasts *Saccharomyces cerevisiae* and *Candida albicans*. In antifungal investigations, Singh *et al.* (2006) found that the dill essential oil was highly effective for controlling the growth of *Penicillium citrinum* and *Aspergillus niger*. In antibacterial investigations, the dill acetone extract has shown better activity against *Staphylococcus aureus* and *Bacillus cereus* in comparison with a commercial bactericide, while the dill essential oil has shown better activity against *Pseudomonas aeruginosa*.

Common basil (*Ocimum basilicum* L.) has been extensively utilized in foods as a flavouring agent, in perfumery and medical industries (Telci *et al.*, 2006).

The present study aimed to investigate the antimicrobial effects of some oils extracted from sweet basil and dill against *Bacillus subtilis*, *Alternaria alternata* and *Penicillium expansum* using the disc diffusion method. The main objective of the study was to establish the inhibitory capacity of the two studied essential oils so they can be successfully used for the preservation of food.

Materials and Methods

Plant material

Dill (*Anethum graveolens* L.) and basil (*Ocimum basilicum*) were provided by Banu Maracine Didactical Station of University of Craiova (44°20'0"N, 23°49'0"E) located in Oltenia region of Romania. Plants used in this experiment have not been treated with plant protection products. The amount of essential oil was 2 ml resulted from one kilogram of dill plants, whereas for basil the sample consisted of 5 ml from 2.5 kg of plants.

In order to extract the dill volatile oil, young stalks and leaves have been used, while for the sweet basil volatile oil, plants which had reached to their full maturity have been chosen, including stalks, leaves and inflorescences.

The essential oil extract

The essential oils were extracted through hydrodistillation (Gîrd, 2009) using a NeoClevenger device. Flask extraction device was adapted to be inserted of 50-100 g shredded vegetable and 500-1,000 mL solvent (usually water). The mixture thus formed was subjected to direct flame heating, sieve. When the solvent reached the boiling point of the extraction, the essential oil was distilled and captured in the graduated tube of the apparatus. After being obtained, the oils were preserved within hermetically closed recipients, under refrigeration.

The antimicrobial activity of the extract

The tests regarding the antimicrobial activity have been performed against two mould strains belonging to the species *Alternaria alternata* and *Penicillium expansum*, as well as against a G (+) sporulated bacteria, namely *Bacillus subtilis*. The antimicrobial action of the oils was assessed by the disc diffusion method as described by Hussain *et al.* (2008). The filter discs (6 mm in diameter) were individually soaked with 15 μL of essential oils and placed on the agar plates which were previously inoculated with the tested microorganisms. Both undiluted oils, as well as oils diluted with n-hexane (at 1:1, respectively at 2:1 ratio), were compared with the control (a disc imbibed with n-hexane only, which brought no antimicrobial effect at all). The Petri dishes were kept at 4 °C for 2 h. The plates were incubated at 37 °C for 24 h for bacteria and at 30 °C for 48 h for fungal strains.

Statistical data

Antimicrobial activity was evaluated by measuring the diameter of the growth inhibition zones in millimetres (including disc diameter of 6 mm) for the test organisms and comparing to the control. A zone of "no growth" around the disk defined the extent of antimicrobial activity. The measurements of inhibition zones were carried for three sample replications, and values were calculated as the average of three replicates.

Results and Discussion

The influence of dill oil against the Bacillus subtilis strain

The best effect against the *Bacillus subtilis* strain has occurred when the essential oil of dill was undiluted. As the oil's concentration decreased, the *Bacillus* colonies increased, which indicated that the strain were resistant at diluted oil. After a thermostatical conditioning of 7 days, it was found that for the cases of undiluted oil and for 1:1 dilution, the inhibition zone remained clean, while at the 2:1 dilution the colonies grew up on a very wide area (Table 1, Figs. 1 and 2).

When the mould from the *Alternaria* species was tested, it was noted that dill volatile oil has acted in an efficient way only undiluted. As the oil's concentration decreased, the strain becomes resistant. The 1:1 dilution with n-hexane determinate a particularly low inhibitory level, while in the case of 2:1 dilution, the colonies grow up even on the disc imbibed with oil. The diameters of the inhibition areas are presented in Table 1 and Figs. 3, 4.

Table 1. The diameters of the inhibition areas determined by the undiluted and diluted dill oil for the studied species

Species / Concentration	Undiluted oil	1:1	2:1	n-hexane
	Diameters (cm) of the inhibition zones			
Bacillus subtillis	2.7	2.5	2.2	Susceptible
Alternaria alternata	2.9	1.5	Susceptible	Susceptible
Penicillium expansum	2.0	1.2	Susceptible	Susceptible

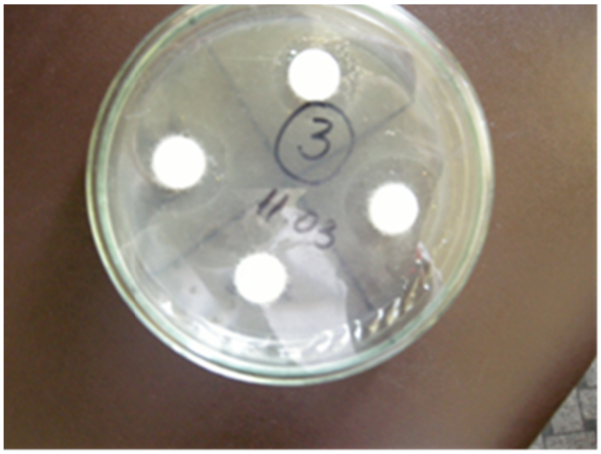

Fig. 1. Diameters of the inhibiting zones at *Bacillus subtillis*

Fig. 2. Diameters of the inhibiting zones at *Bacillus subtillis* (reverse colonies)

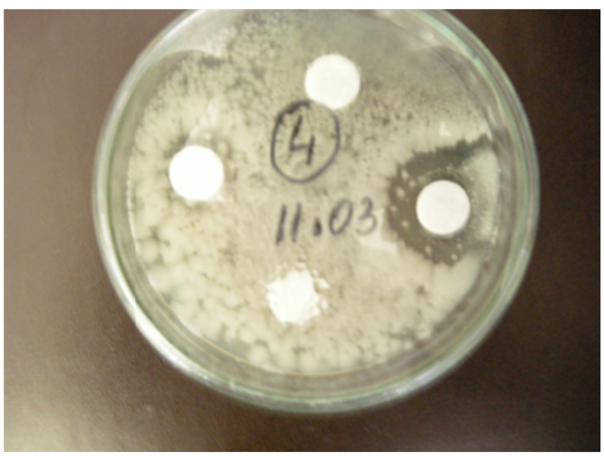

Fig. 3. Diameters of the inhibiting zones at *Alternaria alternata*

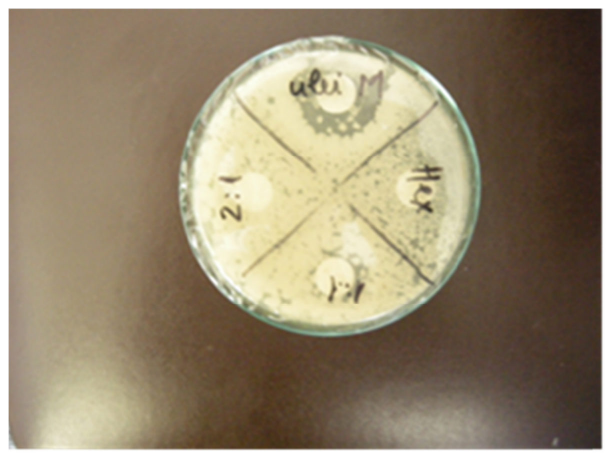

Fig. 4. Diameters of the inhibiting zones at *Alternaria alternata* (reverse colonies)

The present results showed that the oil's dilution led to a decrease by half of its inhibitory strength.

In regard to the *Penicillium expansum* mould, the dill oil had a poor inhibitory action and was effective only when was undiluted.

From the images above it was noted that in the case of the n-hexane's presence, the colonies have come to develop even on the disc surface, while in the case of discs imbibed with oil, the colonies were able to develop till near the disc.

Thus, the present findings showed a good inhibitory activity of dill volatile oil against *Bacillus* species. A hierarchy was established among the tested species using as criterion their susceptibility towards the dill oil's action, as follows: *Penicillium expansum* > *Alternaria alternata* > *Bacillus subtilis*.

The dill (*Anethum graveolens* L.) contains 2.5%-4% volatile oil within its stalks and young leaves, as well as 1%-8% within its fruits (Burzo, 2015). Nineteen different compounds have been detected within the volatile dill oil (Burzo, 2015). In addition, Delaquis *et al.* (2002) reported that dill oil consist approximately equal volumes of carvone and D-limonene, which together accounted for 97.5% of the compounds identified by gas chromatography/mass spectroscopy.

The influence of sweet basil oil

The sweet basil oil has proven to be highly effective when acting against the *Bacillus* strain. The effect of this oil against the tested bacterium was microbicidal, because the strain was inhibited from the whole concerned plate' sector where the oil

Table 2. The diameters of the inhibition areas determined by the undiluted and diluted basil oil for the studied species

Concentration / Species	undiluted oil	1:1	2:1	n-hexane
	Diameters (cm) of the inhibiting zones			
Bacillus subtillis	total susceptible	total susceptible	total susceptible	total susceptible
Alternaria alternata	total susceptible	total susceptible	total susceptible	total susceptible
Penicillium expansum	total susceptible	3.5	3.0	susceptible

was applied, both when oil was undiluted, as well as when it was diluted with n-hexane at a 1:1 ratio. By volatilization, at the temperature of 25 °C, the sweet basil oil produced a strong antimicrobial effect, even in control disc, in which it was noticed a small development of colonies comparing with the dill oil. The same microbicidal effect was found against the *Alternaria alternata* strain. The mould of *Penicillium* species has shown a stronger resistance against the action of the sweet basil oil and in the area where the disc was imbibed with n-hexane only, the colonies were resistant. In the case of undiluted oil, the colonies were susceptible, while in cases of the two dilutions, 1:1 and 2:1, the area of inhibition decreased as the dilution's ratio increased (Table 2).

Basil essential oils and their principal constituents were found to exhibit antimicrobial activity against a wide range of Gram-negative and Gram-positive bacteria, yeast and mould (Suppakul *et al.*, 2003). In addition, Lv *et al.* (2011) reported that the essential oil of basil was active against the Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*).

Hussain *et al.* (2002) assessed the antimicrobial activity of the essential oils obtained from *O. basilicum* against bacterial strains *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pasteurella multocida* and pathogenic fungi *Aspergillus niger*, *Mucor mucedo*, *Fusarium solani*, *Botryodiplodia theobromae* and *Rhizopus solani*. They found that the essential oils of *O. basilicum* exhibited strong antimicrobial activity against all the microorganisms tested, but *S. aureus* and *B. subtilis* were the most sensitive microorganisms, while less activity was observed against *M. mucedo*. In addition, the authors noted that *O. basilicum* essential oil showed greater activity against bacterial strains than fungal strains and it possessed stronger antimicrobial activity against Gram-positive bacteria versus Gram-negative bacteria.

The present findings are in accordance with the above mentioned studies and represent adding data to those obtained by many researchers. Further studies will focus on the combined effect of the tested oils and their interaction with certain compounds resulting from food's fermentation (lactic acid, ethyl alcohol, acetic acid).

Conclusions

The dill oil obtained through distillation procedures had a good antimicrobial effect against *Bacillus subtilis* species and was more effective against *Alternaria alternata* species than against *Penicillium expansum* species. The same hierarchy of inhibition has been confirmed for sweet basil oil, too. The sweet basil oil has proven to be highly effective when acting against the *Bacillus* strain, its effect being microbicidal.

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