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Antimicrobial Activity of Essential Oils from Plants against Selected Microorganisms

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ABSTRACT

Background: it is known that plants have many potential benefits for human health. The purpose of this study was to investigate the antimicrobial properties of several commonly used herbs against specific microorganisms responsible for disease and food spoilage. Essential oils of yarrow, fennel, juniper and marjoram were evaluated, with respect to their efficacy at controlling the growth and survival of several common bacterial and fungal microorganisms.

Methods*:* The agar diffusion test was used to test the essential oils efficacy at inhibiting microbial growth. The following microorganisms were tested with varied results: *Escherichia coli*, *Aspergillus niger*, *Enterococcus faecalis*, *Salmonella typhimurium*, *Salmonella anatum*, *Staphylococcus aureus*, *Candida albicans*, *Penicillium glaucum* and *Bacillus cereus*.

Results*:* Marjoram showed the highest inhibition with a zone up to 14 mm. Juniper and fennel essential oils were also highly inhibitory to *Candida albicans* and the other tested bacteria and fungi.

Conclusion**:** In conclusion, some of the oils were highly effective at inhibiting the studied microorganisms. This knowledge may be useful for further examining the efficacy of pathogenic prevention and food preservation by studied essential oils.

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Introduction

Essential oils (EOs) are liquid mixtures of unstable compounds derived from plants, usually by the steam distillation method (1-4). Historically, people used a wide variety of herbs and EOs because of the health benefits that were well documented in the ancient records (5). Recent scientific studies have proved some of the beneficial characteristics of the EOs, for example, antibacterial, antioxidant, and anti-inflammatory activity (6). Significant numbers of compounds with a low temperature of evaporation have been identified in EOs, and the enormous chemical diversity of their components affects the oxidative stability of EOs (7). Moreover, some essential oils can inhibit the reaction of oxidation, which can be used to preserve other substances, such as food and beverage from having a rank, unpleasant smell or taste (8). As it was mentioned above, several EOs have been found to have an excellent antimicrobial activity. It also performs a central role in some of EOs' biological activities, which is supported by the involvement of medical applications (9).

From the other side, reports on EOs' antimicrobial activity from diverse scientific areas or even laboratories might be controversial, often because of different and specific research methods, which make challenging any correlation among the results. It appears that sometimes a particular procedure used to determine EOs' antimicrobial activity suffers from limitations. In addition, with time, the antifungal activity of the essential oils decreases, most probably due to the evaporation of the volatile compounds (10). Therefore, it is crucial to have the results in a variety of data: in other words, data that are not depends on the specific investigation method. The purposes of the research are to examine the antimicrobial potential of several EOs and also to contribute to current knowledge about EOs, highlighting their potential and value for the application in food industry.

Material and methods

Essential Oils Isolation

Plant materials were selected according to their frequent use in aromatherapy procedures (11). Aerial parts of plants *Achillea millefolium* (Yarrow), *Foeniculum vulgare* (Fennel), *Juniperus communis* (Juniper) and *Origanum majorana* (Marjoram) were collected in different parts of Romania during harvest season in 2016, air-dried and were kept in the dark till the distillation takes place.

The essential oils were isolated at the chemical laboratory at the Faculty of Agriculture, Science, Food Industry and Environmental Protection at "Lucian Blaga" University of Sibiu, Romania. Steam distillation method was used to obtain the essential oils from air-dried plant materials. EOs were stored in dark bottles and kept refrigerated until evaluation. The quantities of the EOs were determined gravimetrically.

Culture maintenance and inoculum preparation

The microorganisms used were as follows: *Escherichia coli*, *Enterococcus faecalis*, *Bacillus cereus*, *Salmonella typhimurium*, *Salmonella anatum*, *Staphylococcus aureus*, *Candida albicans*, *Penicillium glaucum*, *Aspergillus niger*. All strains were obtained from the Culture Collection of the microbiological laboratory at the Faculty of Agriculture, Science, Food Industry and Environmental Protection at "Lucian Blaga" University of Sibiu, Romania. Organisms were maintained on blood agar (BA). Overnight cultures were prepared by inoculating approximately 2 ml Mueller Hinton broth (MHB) with 2–3 colonies of each organism taken from BA. Broths were incubated overnight at 35 °C with shaking. Test strains of bacteria were inoculated onto Plate Count Agar (PCA) and MacConkey agar (MCA) for *Escherichia coli*. *Candida albicans, Penicillium glaucum and* and *Aspergillus niger* were maintained on yeast and mould agar and revived on yeast and mould broth at 25°C.

Microorganisms were incubated at 35°C for 72 h. All cultures were kept in the dark during the experiment (up to 2 weeks) and were visually analyzed for purity before each use.

Assay for antimicrobial properties

For the evaluation of the antimicrobial activity of the essential oils, the filter paper disc diffusion method with slight modifications was used [3]. PCA, MCA and Mueller Hinton agar (MHA) were used for the antibacterial assay. PCA with slight modifications was also used for the antifungal assay. Each plate was inoculated with bacterial or yeasts culture (100 μL) directly from the broth. The quantity of essential oil (100%) applied to each sample was 10 μL. The tested oils were placed on sterile filter paper discs. The diameter of the disk was five millimeters. According to the methodology [3], the disks were placed in the center of the Petri plate. Minimum inhibitory concentrations (MICs) were determined after incubation at 37°C for three days. As the last step in the process, the inhibition zones were measured and documented in millimeters (mm). To avoid possible mistakes, the evaluation of antibacterial activities of essential oils was carried out in three repetitions. The MICs were determined as the lowest concentration of oil inhibiting the visible growth of each organism on the agar plate.

Statistical analysis

The data were analyzed using the analysis of Variance (ANOVA) from the analysis tool package in Microsoft Office Excel 2016. The p-value of 0.05 was set as a limit for a statistically significant difference in the studies.

Results

The EOs yield was approximately 0.7% for each plant material. Antimicrobial activity of examined essential oils against selected pathogens: bacteria, yeasts, and moulds are represented in the tables 1 to 3. It can be seen from the results that antimicrobial performance ranged from no inhibition to complete inhibition (no growth) on the examined organisms.

According to Table 1, there was a complete inhibition of growth of the Gram-positive bacteria *E. faecalis* by the all tested oils. On the contrary, EOs of marjoram and fennel were significantly less effective against *B. cereus* (zones = 12 mm for each) and *S. aureus* (zone = 10 and 8 mm respectively). Even though plates with yarrow showed some inhibition zones (6 and 10 mm) against *B. cereus* and *S. aureus*, it was slightly less active compared to other EOs' effect on Gram-positive bacteria. Juniper was also moderately effective against *B. cereus* and *S. aureus*.

As it is shown in Table 2, all the tested Gram-negative bacteria *S. typhimurium*, *S. anatum* and *E. coli* were inhibited by the marjoram EO, and the greatest result was estimated for *E. coli*, where the inhibition zone is 14 mm. These data confirm the previous study (12) which suggests that the EO of *O. majorana* possess antimicrobial properties. Also, the research managed by (13) reports that the EO of marjoram have a great potential against *Salmonella* strains and other pathogens. Fennel EO as it was published by (14) was quietly effective against selected microorganisms, but in the present study didn't have any effect on *S. typhimurium*. It might perform better antibacterial activity in combination with others antimicrobials (15, 16). Stable inhibition of growth by yarrow and juniper was found against tested Gram-negative pathogens.

Zones of inhibition for moulds and yeasts (Table 3) ranged from 6 to 14 mm, which represents moderately to strongly inhibitory effect on those microorganisms. *A. niger* appeared more resistant to the all applied essential oils by comparing it with *C. albicans* and *P. glaucumand*. Moreover, yarrow EO showed no inhibitory effect on the growth of *A. niger*.

Table 1. Inhibition of growth of Gram-positive bacteria by essential oils.

Details of plant oils		Test organism		
Plant species	Common name	*Enterococcus faecalis*	*Bacillus cereus*	*Staphylococcus aureus*
		Diameter of inhibition zone (mm)		
Achillea millefolium	Yarrow	-	6^{ac}	10^{ab}
Foeniculum vulgare	Fennel	-	12^{b}	8^{abc}
Juniperus communis	Juniper	-	8^{abc}	5^{ac}
Origanum majorana	Marjoram	-	12^{b}	10^{ab}

* "-" no growth

$^{a-c}$ Means in a column without a common superscript letter differ from each other (P<0.05)

Table 2. Inhibition of growth of Gram-negative bacteria by essential oils.

Details of plant oils		Test organism		
Plant species	Common name	*Salmonella typhimurium*	*Salmonella anatum*	*Escherichia coli*
		Diameter of inhibition zone (mm)		
Achillea millefolium	Yarrow	6^{a}	10^{a}	10^{a}
Foeniculum vulgare	Fennel	0^{b}	10^{a}	7^{ab}
Juniperus communis	Juniper	10^{a}	9^{a}	6^{b}
Origanum majorana	Marjoram	9^{a}	10^{a}	14^{ac}

$^{a-c}$ Means in a column without a common superscript letter differ from each other (P<0.05)

Table 3. Inhibition of growth of fungi and yeasts by essential oils.

Details of plant oils		Test organism		
Plant species	Common name	*Candida albicans*	*Penicillium glaucum*	*Aspergillus niger*
		Diameter of inhibition zone (mm)		
Achillea millefolium	Yarrow	10^{a}	14^{a}	0^{b}
Foeniculum vulgare	Fennel	11^{a}	10^{a}	8^{a}
Juniperus communis	Juniper	8^{a}	0^{b}	6^{a}
Origanum majorana	Marjoram	12^{a}	10^{a}	6^{a}

$^{a-c}$ Means in a column without a common superscript letter differ from each other (P<0.05)

Discussion

The stable antimicrobial activity of marjoram against Gram-positive and Gram-negative pathogens and fungi suggests that marjoram EO reasonably might be beneficial in some food technologies as an antimicrobial agent. Even though the smell of the marjoram EO quite pleasant it might have some limitations in practice for example in foods or beverages where the marjoram flavor would be unappropriated. In addition, the antimicrobial activity of EOs appeared to be remarkably different depending on the conditions of cultivating species of the plant (17). The commercial applications thus may be bounded. From the other side, EOs because of its antimicrobial properties and other advantages over commercial microbicides, may prove to be an effective herbal protectant against a broad variety of pathogenic bacteria and fungi, since they are known as non-toxic and eco-friendly agents (18).

Conclusion

From this study it can be concluded that the activity was more pronounced against Gram-negative and fungal organisms than Gram-positive bacteria. Nevertheless, the mechanisms responsible for the antimicrobial activity are, however, less observed and the much further investigation is required.

Conflict of interest

There is no conflict of interest.

Financial disclosure

The authors declared no financial disclosures.

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