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A study regarding the antibacterial activity of some comercial essential oils on food-borne pathogenic and spoilage bacteria

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Abstract

The aim of this study was to investigate the antibacterial activity of some commercial essential oils, obtained from herbs and spices commonly used in cookery, against a panel of food-borne pathogenic and spoilage bacteria such as ***Stafilococcus aureus***, ***Escherichia coli*** and ***Bacillus cereus***. Eight essential oils were tested (dill oil, juniper oil, oregano oil, basil oil, thyme oil, rosemary oil, fir oil and clove oil) in different concentrations (1 µl, 5µl şi 10µl) using the agar diffusion technique. The results were compared with those of a commercial antibiotic – gentamycin, which was used as a positive control at a constant concentration of 10 µl. Some of the essential oils exhibited promising antibacterial effect as a diameter of zones of inhibition (21-33 mm) against the tested bacteria. The results indicate the potential efficacy of plant-based natural products such as essential oil of oregano, clove and thyme to control food-borne pathogenic and spoilage bacteria.

Keywords: Antibacterial activity, essential oil, agar diffusion, food-borne bacteria

1. Introduction

Food-borne diseases are a growing public health problem worldwide. It is estimated that each year in the United States, 31 species of pathogens cause 9.4 million cases of food-borne illnesses [18]. Successful control of food-borne pathogens requires the use of multiple preservation techniques in the manufacturing and storage of food products. A recent consumer trend toward preference for products with lower salt and sugar content presents an increased need for efficient food preservatives, as lowering the salt and sugar content would otherwise compromise the product's shelf-life[20]. A wide range of preservatives are used to extend the shelf-life of a product by inhibiting microbial growth. However, an increasingly negative consumer perception of synthetic food additives has spurred an interest in finding natural alternatives to the traditional solutions [20].

Although originally added to change or improve taste, the antimicrobial activity of essential oils makes them an attractive choice for substituting synthetic preservatives.

Essential oils are complex mixtures of volatile compounds with strong odor that are synthesized in several plant organs, including buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood or bark, and stored in secretory cells, cavities, canals, epidermic cells or glandular trichomes [2, 8]. These volatile compounds have diverse ecological functions, acting as defensive substances against microorganisms and herbivores, but can also be important to attract insects for the dispersion of pollens and seeds [2].

Essential oils have therapeutic uses in human medicine due to its anticancer, antinociceptive, antiphlogistic, antiviral, antibacterial and antioxidant properties [4].

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Additionally, the use of essential oils is becoming popular to increase the shelf-life of food products, since consumers are more conscious about the health problems caused by several synthetic preservatives [9,10].

Synthetic preservatives that are added in food items, as antimicrobials and antioxidants, are considered to be without potential adverse effects and are classified as generally recognized as safe. However, there have been problems concerning the safety of some chemicals, including the possibility of allergies from benzoic acid and sulphites, the formation of carcinogenic nitrosamines from nitrites, and the possible rodent carcinogenicity of butylated hydroxyanisole and butylated hydroxytoluene [16].

Studies with essential oils as food additives revealed to be advantageous, as observed by the increase in food shelf-life [6]. Still, the amount of essential oils used was determinant for the acceptance, as strong aromas of essential oils might be imparted to food products [6]. Several factors influence the chemical composition of plant essential oils, including the species, part of the plant, season of harvesting, geographical origin, and also the extraction method, and consequently their bioactive properties [2, 11,14,15,17, 19]. The antibacterial and antioxidant properties of many essential oils and constituents have been studied so far. However, most results published so far are dispersed and employed different techniques, making comparison rather difficult. Additionally, several plant essential oils properties have not yet been studied. Therefore, the present study aimed to screen antibacterial properties of 8 commercially available essential oils against 3 food-borne spoilage and pathogenic bacteria.

2. Materials and Methods

Essential oils. The essential oils used in this study were commercial samples. The steam-distilled essential oils of dill, juniper, oregano, basil, thyme, rosemary, fir and clove were purchased from a Plafar store from Cluj Napoca. Dill oil, juniper oil, oregano oil, basil oil, thyme oil, rosemary oil and fir oil were manufactured by *SOLARIS* and the clove oil, by *FARES*. The oils (100% pure essential oil) were stored in dark bottles and kept refrigerated per manufacturer`s recomandation until evaluation.

Strains and culture media. The essential oils were tested on the following strains: *Escherichia coli* ATCC 11229, *Staphylococcus aureus* ATCC 6538P and *Bacillus cereus* ATCC 11778. E. coli was cultured on Tryptone Bile X-glucuronide Agar (TBX), S. aureus was cultured on Baird–Parker agar (BP) and B. cereus was cultured on Mannitol Egg Yolk Polymyxin Agar (MYP).

Standardisation of inoculums. Pellets of pure culture of the test bacteria were inoculated in sterilized nutrient broth. After incubation at 37^{o}C the absorbance of the bacteria was read taking the nutrient broth as control.

Antibacterial and antifungal assay. The antibacterial activity was evaluated as in traditional antibiotic susceptibility testing using the disc diffusion method [3,7,12,13]. The appropriate solidified medium was inoculated with bacterial inoculum and spred over the plates using a sterile cotton rod, display in order to get a uniform microbial growth. After inoculum absorbtion by agar, 10 sterile filter discs (handmade of 0,3 mm thick filter paper, 6 mm diameter) were placed on the agar surface using forceps dipped in ethanol and flamed: 8 for the essential oils, 1 for the positive control- gentamycin solution (10µL/ml) and 1 for the negative control- physiological serum.

Three dilutions of stock solutions of essential oils were tested for each microorganism (1 µl, 5 µl and 10 µl-each dilution on a different petri dish). In order to compare the efficiency of the tested essential oils 10 µl of the pozitiv as well as of the negative control were used on all of the cases.

After the discs were impregnated with all the tested solutionsand essential oils, the dishes were left for 30 min at room temperature to allow the diffusion of oil, and then were incubated at 35-37^{0}C for 24 hours.

After the incubation period, the mean diameter of inhibition halo where test microorganism did not grow (clearly visible inhibition zone) was measured in millimeters.

3. Results and discussion

The The antimicrobial activity of the selected essential oils against three bacterial species was qualitatively assessed by the presence or absence of the inhibition zone. For *Escherichia coli* and *Staphylococcus aureus* the inhibition zones were well defined and easily measured.

Due to the opacity of the culture medium, for *Bacillus cereus*, the halos were a little blurry but measurable. The antibacterial activity is summarized in Table 1.

Table 1. Antibacterial activity of tested essential oils

Essential oils	**Tested bacteria**								
	Escherichia coli			*Staphylococcus aureus*			*Bacillus cereus*		
	1 µl	5 µl	10 µl	1 µl	5 µl	10 µl	1 µl	5 µl	10 µl
	Mean inhibition zone diameter (mm)* after 24 h of incubation								
Dill	8.5	15.5	24	7	11	19.5	7	7.5	11
Juniper	0	0	25	5.5	7	10.5	6	8	9
Oregano	25.5	30	33	21	31	31.5	23.5	23.5	23.5
Basil	7.5	12	37	6	16	19	0	12.5	13
Thyme	16	16.5	20	15	22.5	28.5	12	14.5	17
Rosemary	0	0	0	5.5	8.5	10.5	0	7	8.5
Fir	0	7	7.5	5.5	8.5	10.5	0	7.5	9
Clove	15	17	20	15.5	22.5	26.5	10.5	16.5	23
Reference control Gentamycin 10µL/ml	**14**	**14**	**14.5** **	**22.5**	**19**	**17** **	**15**	**17.5**	**17** **

* The diameter of the filter paper disc (6 mm) is not included.
**10 µl of the reference control was used every time

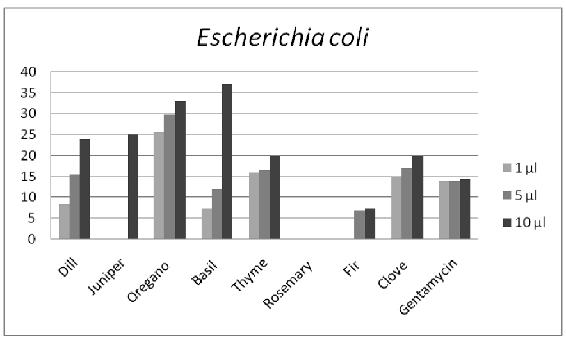

***Figure 1**.* Grafical representation of the results obtained for e. coli

In the case of Escherichia coli, as figure 1 shows, the most effective antibacterial activity was exhibited by the basil essential oil at 10 µl, but the same essential oil at lower quantities had a significantly lower effect. Oregano essential oil had a higher antibacterial activity than the gentamycin even used at lower quantities.

The essential oil of rosemary had no effect at all on e. coli and the juniper essential oil showed antibacterial activity only used at 10 µl.

For most of the essential oils, the antibacterial activity does not increase significantly by increasing the quantity of the oil.

Regarding staphylococcus aureus, as showed in figure 2, the oregano essential oil is the most effective at all three levels with no significant differences from 5 µl to 10 µl.

The rosemary, fir and juniper essential oils exhibited, even at 10 µl, lower antibacterial activity than gentamycin used in same quantity. Also the increase quantities of essential oils showed an increase in the antibacterial activity but still not proportionately.

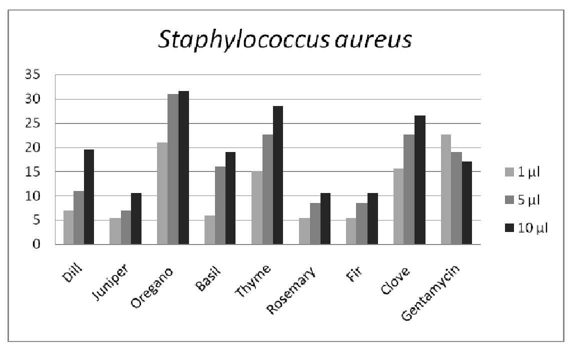

***Figure 2**.* Grafical representation of the results obtained for s. aureus

As showed in figure 3, again, the highest antibacterial activity, in the case of bacillus cereus, was registered by the oregano essential oil.

At all three quantities used, the inhibition zone of oregano essential oil was the same, measuring 23.5 mm, larger than the inhibition zone created by gentamyicin. At 1 µl the basil, rosemary and fir essential oil had no antibacterial activity against b.cereus and lover antibacterial activity than gentamycin even at 10 µl.

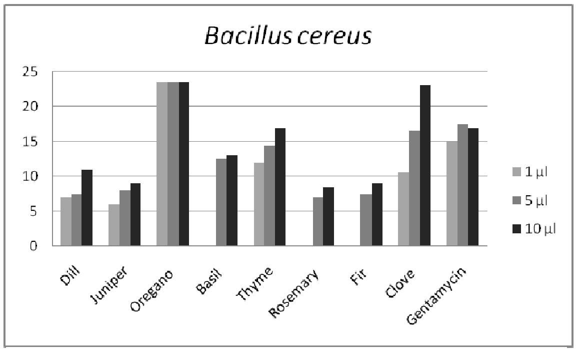

Figure 3. Grafical representation of the results obtained for b. cereus

The probable mechanisms of action of the essential oils on bacteria are given by their hidrophobicity, which enable them to partition the lipids of the bacterial cell membrane and mitochondria, action that cause the structural damage or complete rupture of the cellular membranes, losses of nutrients and increased permeability [5].

4. Conclusion

The results revealed that the selected essential oils showed significant antibacterial activity. Among the essential oils, oregano, clove and thyme oil exhibited the most effective antibacterial activity against all of the tested bacteria, with inhibition zones reaching 33mm, 26.5mm and 28.5mm, having a greater inhibition diameter than the control sample (gentamycin 10 µL/ml) even when used in smaller doses. At the opposite pole, the least effective antibacterial activity was showed by the rosemary essential oil, that had no effect on *Escherichia coli* at neither of the used quantities, some little effect on *Bacillus cereus* at 5 and 10 µl and on *Staphylococcus aureus,*compared to the reference control, had a smaller effect.

The results are encouraging and consistent to other articles found in literature - Alina Dobre found in found in her study similar results [1]. These results open a new horizon of laboratory research for an alternative to prevent the development of germs in food and their conservation without endangering human health especially since the chemical residues accumulation is excluded.

Further studies are needed on incorporation of these oils into appropieate food formulations to evaluate flavor, chemical changes, and antimicrobial effect in a food system. Some essential oil were highly inhibitory to pathogens at low levels, but it has to be emphasized that these oils may not be useful as preservatives in food that will receive heat treatment after the addition of essential oil because it is likely that the antimicrobial compounds would be evaporated.

All that been said, practical applications of the results obtained herein are predicted.

Compliance with Ethics Requirements

Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

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