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INHIBITORY ACTIVITY OF ESSENTIAL OILS ON ESCHERICHIA COLI

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Abstract

Bacteria are prokaryotic, single-celled microorganisms, among the first life forms to appear on Earth, and are present in most existing habitats. Bacteria are a group of microorganisms with a wide spread in nature as a result of their adaptation during the evolutionary process. Escherichia coli is a Gram-negative, short bacillus that does not form spores; it is mobile, usually with peritric flagella, forming individual colonies or pairs. The genus Escherichia is part of the large Enterobacteriaceae family, having as a habitat, largely, the intestines of humans and animals. Because some strains cause serious infections and are resistant to conventional treatments, herbal extracts are studied and used, at least as an adjuvant, in the treatment of these conditions in humans and domestic animals. Herbal essential oils are increasingly popular, being used both in perfumery, cosmetics, and natural medicine industries. Currently, there are numerous scientific studies that have experienced the properties of these oils. In this research, the inhibitory effect of 19 essential oils on Escherichia coli was studied. The method used was the Kirby-Bauer diffusion method, often used in microbiology laboratories to test the antibiotic sensitivity of various bacteria. The reading of the results was achieved by measuring the inhibition zone in mm using a ruler, including the disc diameter (6 mm). The essential oils used were purchased commercially and the bacteria tested was Escherichia coli, a resource obtained from the "Horia Cernescu" Research Laboratory Complex of the "King Michael I of Romania" Banat University of Agricultural Sciences and Veterinary Medicine in Timisoara, Romania. The results obtained show that Escherichia coli is highly sensitive to oregano (34 mm), wild thyme (32 mm), garden thyme (27 mm), rosemary (25mm), tea tree (22 mm), and clove (21 mm) oils. It is very sensitive when treated with cinnamon oil (17 mm), peppermint (15 mm), and basil (15 mm), and sensitive when treated with lavander (10 mm), fennel (9 mm), dill (9 mm), garden sage (9 mm), and cumin (9 mm) oils. It is non-sensitive when treated with patchouli (0 mm), grapefruit (8 mm), vervain (8 mm), black cumin (7 mm), and rose geranium (7 mm) oils.

• Introduction

E. coli is a Gram-negative, short bacillus that does not form spores; it is mobile, usually with peritric flagella, forming colonies individually or in pairs, capable of fermentative and respiratory metabolism. *E. coli* remains one of the most common causes of many bacterial infections, such as enteritis, urinary tract infection, septicaemia or clinical infections, as well as neonatal meningitis.

Essential oils are natural products derived from medicinal, aromatic plants, traditionally used worldwide for disinfection, as anti-inflammatory, antibacterial and antifungal substances, relaxing and stimulating, with potential for use in clinical medicine.

The main objective of this study is to test some commercially obtained essential oils on *E. coli* bacteria to verify the veracity of these products and their effect on *in vitro* bacterial cultures.

• Material and method

The bacterium used to test sensitivity to essential oils is *E. coli* obtained from the "Horia Cernescu Research Laboratory Complex" at the University of Agricultural Sciences and Veterinary Medicine of Banat "King Michael I of Romania" in Timisoara, Romania. The essential oils used were bought from naturist pharmacies, from different manufacturers.

Kirby-Bauer diffusion method

- The culture medium used was agar supplemented with blood (BAB 5%) in Petri boxes. A tube containing MHB inoculated with the microorganism was incubated during 18 h, at 37°C. Decimal dilutions were made. 200 µl of inoculum were spread over Petri plates. Subsequently, the discs were added After fixing the discs, the essential oils were added, 10 microliters on each disc. The plates were left for 15 minutes at room temperature to allow the oil diffusion; they were kept in the incubator for 24 hours at 37°C. After this time, the antibacterial activity was evaluated by measuring the diameter of inhibitory zones around the discs using a calliper.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC).

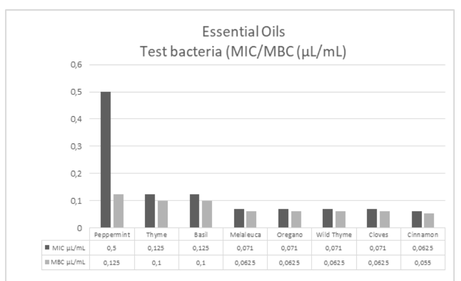
The bacterium was grown in Mueller Hinton Broth at 37°C for 18-24 h. In sterile tubes with MHB, decimal dilutions were achieved with each essential oil after which the bacterial suspension was inserted into each tube to result in a final volume of 4 ml. Final solutions were incubated at 37°C overnight. The MIC was defined as the lowest concentration that inhibited visible growth. The MBC was determined by subculturing 100 μL from the negative test tube onto plates (BAB 5%). MBC was defined as the lowest concentration resulting in a negative subculture.

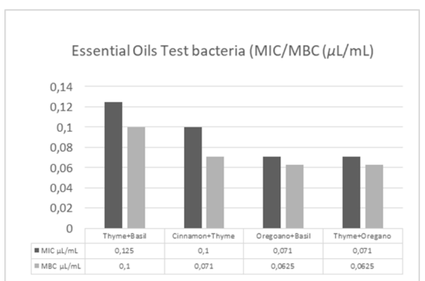
Interaction between Components of Essential Oils

Based on the information in literature on the biochemical composition of essential oils and the existence of synergistic relationships between them, we tested, by the same Kirby-Bauer diffusion method, the antibacterial effect produced by the combinations between 2 essential oils, selected after the results obtained in the first stage of the study.

• Results and discussions

Minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC)





MIC represents the lowest concentration that can inhibit the growth of the bacteria. For this, essential oils were selected that had a higher antibacterial effect in the first stage of the study.

The MBC can be explained as the µL quantity of the essential oil that we need for the inhibition of *E. coli*.

Four combinations made from 2 essential oils were also tested. The combination between essential oils oregano / basil and oregano / thyme inhibits the bacteria at a lower concentration than the combination between cinnamon / thyme and thyme / basil.

Interaction between components of essential oils

Thymol / Eugenol (thyme & basil) | Cinnamaldehyde / Thymol (cinnamon & thyme)

Carvacrol / Eugenol (oregano & basil) | Thymol / Carvacrol (thyme and oregano)

Essential oils	Inhibition zone (mm)
Carvacrol / Eugenol	30
Thymol / Carvacrol	25
Thymol / Eugenol	23
Cinnamaldehyde / Thymol	15

In the case of oils tested by us also, the combination of phenols (thymol with carvacrol, and both components with eugenol) were synergistically active against *E. coli* strains (BASSOLE & JULIANI, 2012).

Antimicrobial activity of essential oils

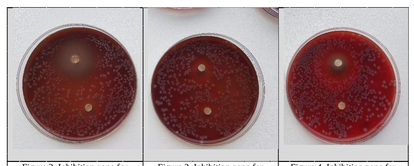


Figure 2. Inhibition zone for oregano and basil oils

Figure 3. Inhibition zone for melaleuca and peppermint oils

Figure 4. Inhibition zone for rosemary and lavender oils

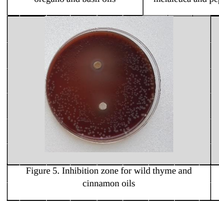


Figure 5. Inhibition zone for wild thyme and cinnamon oils

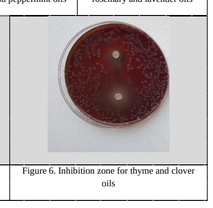


Figure 6. Inhibition zone for thyme and clover oils

Extremely sensitive: Inhibition zone >20 mm	Very sensitive: Inhibition zone 15-19 mm	Sensitive: Inhibition zone 9-14 mm	Non-sensitive: Inhibition zone <8 mm

Essential oils	Inhibition zone (mm)	Essential oils	Inhibition zone (mm)
Oregano	34	Fennel	9
Wild thyme	32	Dill	9
Thyme	27	Garden sage	9
Rosemary	25	Cumin	9
Melaleuca	22	Grapefruit	8
Cloves	21	Vervain	8
Cinnamon	17	Geranium	7
Peppermint	15	Black cumin	7
Basil	15	Patchouli	0
Lavander	10		

For oils that produced an inhibition area diameter of more than 20 mm, *E. coli* was considered to be extremely sensitive; for values between 15 and 19 mm, we characterized it as very sensitive; and, for diameters between 9 and 14 mm, it was called sensitive; for all values below 8 mm, *E. coli* was considered not sensitive.

•Conclusions

Our study verified the inhibitory effect of commercially available essential oils against *E. coli*. Numerous essential oils showed medium to high antibacterial activity, with very good results in oregano, thyme, wild thyme, rosemary, melaleuca, and cloves.

Patchouli essential oil, a species proven to have antibacterial action for *E. coli*, was the only one that had no inhibitory effect at all, which means that some commercially available essential oils are not genuine, especially common in those obtained from exotic species.

Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC), together with tests performed with combinations of two oils, have confirmed oregano essential oil as the most effective of all those tested by us.

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