THE COMPARATIVE ANTIMICROBIAL EFFECTS OF *OCIMUM BASILICUM* ESSENTIAL OIL IN A PURE FORM AND EXPOSED TO AN ELECTRIC FIELD

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Abstract. Lately, essential oils have started to receive an increased attention both from the prime consumers and from the scientific community because people are beginning to be more interested to invest in a healthy lifestyle. Their complex composition grants them multiple effects including antibacterial, antiviral and antifungal effects, which led them to be envisioned as a valuable alternative for the extensive use of antibiotics, since bacteria started to develop resistance and become more difficult to treat. In this study we compared the antibacterial and antifungal effects of basil essential oil and basil essential oil exposed to an electric field on six bacterial strains and one strain of *C. albicans*. To meet our objective, we've used the agar diffusion method. Our results showed that basil essential oil has a mild inhibitory effect on most of the bacteria and on C. albicans. Additionally, the basil essential oil exposed to the electric field mostly kept the same profile, even though it proved that it caused a slight decrease in the values of the inhibition potential of basil essential oil, except for S. enteritidis, where it showed an increase in its antibiotic capacity.

Keywords: Basil (*Ocimum basilicum*) Essential Oil, antibacterial, antifungal effects, *Candida albicans,* agar diffusion test, electric field exposure

INTRODUCTION

In the last years there has been an increase in the demand of essential oils as people became more aware of the need to consume high quality, natural, organic products. A research report by Facts and Factors estimated the global essential oils market at USD 7 Billion in 2019 and was expecting it to reach a double value by 2026. Essential oils cover a wide range of uses from gastronomy, medical, pharmaceutical, perfumery, house cleaning and spa and relaxation industry.

According to The Essential Oils Desk Reference, the 7th edition, essential oils are aromatic, volatile liquids extracted from different parts of the plant such as buds, twigs, flowers, seeds, fruits, roots, leaves, stems, wood or bark that are stored in secretory cells, cavities, canals, epidermic cells or glandular trichomes of the specific plant. Some of the extraction techniques include water or steam distillation, expression under pressure, solvent extraction, supercritical fluid extraction and subcritical water extraction (Vostinaru et al., 2020).

The Essential Oils Desk Reference, the 7th edition mentions that essential oils are mixtures of volatile organic compounds such as hydrocarbons (terpenes and sesquiterpenes) and oxygen-containing substances (alcohols, esters, ethers, aldehydes,

ketones, lactones, phenols and ether-phenols). The composition of essential oils is complex and it asks for a proper attention. A single essential oil may contain from 80 to 300 or more constituents, which requires a variety of methods for the full understanding of their effects and mechanisms of action.

According to the same Essential Oils Desk Reference, there are numerous studies that reveal different therapeutic actions of essential oils such as: antiviral, antibacterial, antifungal, repellent, analgesics, anti-inflammatory, antitumor, immunomodulatory, sedative, spasmolytic, local analgesics, modulators of genetic expression and influencing cell signaling pathways, antioxidant, anxiolytic, etc.

Sweet Basil (*Ocimum basilicum*) belongs to *Liliaceae* family which comprises over 150 species. It has been known and widely used in traditional medicine, especially in the Asian-Indian area. Basil received the name of "the King of herbs" due to its rich composition which is responsible for its antiviral, antibacterial, anti-inflammatory, muscle relaxant and powerful spasmodic effects. In the Indian tradition it was highly valued for its spiritual and religious properties as people believed that it spread positive energy in the environment. Also, due to its healing effects it was used in anxiety, sickness, migraines, throat and lung infections, insect bites, acne, mental fatigue, digestive disorders and kidney malfunction. Currently, sweet basil it is widely cultivated as an ornamental plant as well for the extraction of essential oil. In the Mediterranean gastronomy it is used as an ingredient in the preparation of many dishes (Semeniuc et al., 2017 and Essential Oils Desk Reference).

More authors, including (Socaci et al., 2008) and (Semeniuc et al., 2017) state that the chemical composition of essential oils is influenced by the climate, the environmental growth conditions, the agronomic techniques of cultivation and extraction methods.

MS chromatography analyses determined that the main constituents of basil essential oils contained monoterpenes derivatives (camphor, limonene, 1, 8-cineole, linalool, geraniol) and phenyl propanoid derivatives (eugenol, methyl eugenol, chavicol, estragole, methyl-cinnamate) Different chemotypes of basil have been recognized based on the predominant essential oil constituents (e. g. linalool, methyl chavicol, methyl cinnamate, methyl, eugenol, eugenol) (Semeniuc et al., 2017).

Due to the high increase of antibiotic resistance new solutions more natural and in tune with the human biology are investigated. Thus, there is an increase interest in the study of antimicrobial effects of the essential oils against various bacterial and fungal strains, including the ones of basil essential oil.

Moreover, several studies show that electrical stimulation of different types of seeds can induce a simulative or inhibitory effect on various seed germination (Sidaway G. H., 1966), (Li Z.-G et al., 2019). Such results made us wonder if the electrical stimulation may present a similar effect on the microbiological activity of essential oils (Liu Q et. Al, 2017).

Thus, in order to further research the antibacterial and antifungal effects of basil (*Ocimum basilicum)* Essential Oil (EO) we have conducted a comparative study between the effects of pure basil EO and basil EO energized in an electric field on different Gram-positive and Gram-negative bacterial strains and one Candida culture along with a selected antibiotic/antifungal of reference.

MATERIAL AND METHODS

1. Essential oil used

For this study we have used Basil (*Ocimum basilicum*) Essential Oil from a renowned company from the market.

2. Electrical stimulation the essential oil

The sample consists of 2 ml of basil essential oil which was stimulated by using a flat capacitor type device which has the possibility to influence the distance between the armatures and the feeding tension, as well.

The applied tension was of 25V of and lasted for a period of 20 minutes.

3. Bacterial strains

Six bacterial strains were used in this study as follows: three Gram-positive bacterial strain *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 6538P), *Listeria monocytogenes* (ATCC 13932), three Gram-negative strains *Escherichia coli* (ATCC10536), and *Salmonella enteritidis* (ATCC 13076), *Pseudomonas aeruginosa* (ATCC 27853) and one yeast *Candida albicans* (ATCC 90028).

4. Agar diffusion susceptibility testing

EOs samples were assessed against all bacteria and *Candida* using the agar diffusion method (5-mm sterile paper disks). Amoxycillin-clavulanic acid 30 (20+10) µg, Enrofloxacin 5 µg and Nystatin 100IU were used as controls for Gram positive, Gram negative and fungal strain, respectively.

Each strain was grown in a test tube containing 1-2 mL sterile nutrient broth (Oxoid Ltd., Basingstoke, Hampshire, England) at 37 C for 24 hours. The purity of the inoculum was confirmed by plating on appropriate selective media and microscopic ex-amination of the Gram-stained smear (Optika microscope, B-252, M.A.D; Apparecchiature Scientifiche, Milan, Italy). One hundred µL of inoculum was dispersed over the entire surface of the Mueller-Hinton agar plate and was incubated at 37 C for 24 hours. *Candida albicans* was inoculated on a Sabouraud agar plate. Out of these, a small quantity of bacteria from an isolated colony was taken with the loop and was put in a tube with 2 ml sterile saline solution, in order to obtain a homogenized suspension with the density of 0.5 on McFarland scale.

Then, 1 ml of suspension was evenly distributed on the entire surface of a Petri dish. The excess was removed and the plaques with their lids semi-opened were put in a dry a laminar flow cabinet near the gas bulb.

Out of these plates our study dilutions were prepared. A sterile paper disk of 0.5 mm was placed in the middle of a Petri dish. On each paper we put 5 µL of EO from each sample. Plates were incubated for 24 hours at 37 C. A digital caliper was used to measure the inhibition zone diameter (in millimeters).

RESULTS AND DISCUSSIONS

The antibacterial effect of the studied sampled was assesed by using the agar diffusion method. The scale that was used to interpret the results was as follows (disk diameter - 5mm - included): strong inhibitory effect: zone of inhibition > 28 mm,

moderate inhibitory effect: 16 mm < zone of inhibition < 28 mm, mild inhibitory effect: 9 < zone of inhibition < 16 mm, and no inhibitory effect: zone of inhibition < 9 mm (Semeniuc et al., 2017).

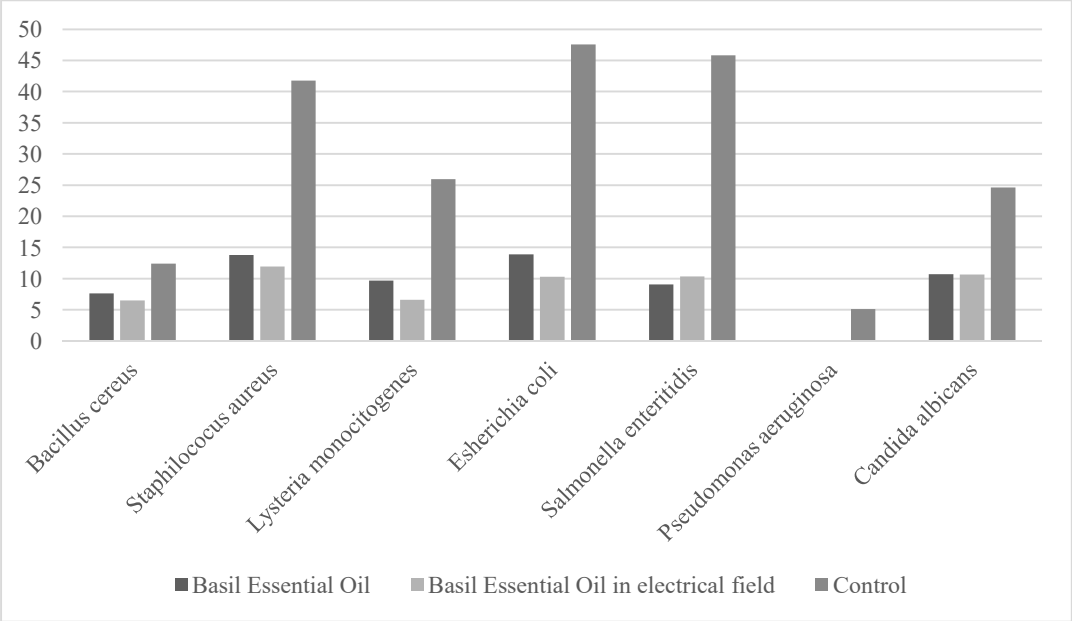

Fig. 1. The antimicrobial activity of pure basil essential oil comparative with basil essential oil exposed to an electric field, at a dose of 5 µL, on different bacterial strains and one culture of *Candida albicans*

Many studies have highlighted that Basil essential Oil showed antibacterial effects on the selected strains. For example, a study by Semeniuc CA et al., 2017, revealed that basil essential oil had a mild inhibitory effect on all the investigated bacterial strains: *B. cereus* (ATCC11778), *S. aureus* (ATCC 6538P), *P. aeruginosa* (ATCC 27853), *E. coli* (ATCC 25922), and *S. typhimurium* (ATCC 14028).

In our study, basil EO showed a similar mild inhibitory effect, according to the above-mentioned scale, on *S. aureus, L. monocytogenes, E. coli, S. enteritidis*, and on one investigated fungus, *C. albicans*, and proved to have no inhibitory effect on *B. cereus*. The inhibition zones varied from 7.61 to 13.88 mm.

The effects of basil EO submitted to an electric field were as follows: on *B. cereus* and L. *monocytogenes* there were no inhibitory effect, while on *S. aureus, E. coli, S. enteritidis* it showed a mild inhibitory effect. The inhibition zone range was between 6.49 and 11.96 mm.

Both analyzed essential oils showed similar values on *C. albicans* with a mild inhibitory effect of 10.65 mm, while *P. aeruginosa* proved to be resistant to the both types of samples.

When compared with the pure essential oil, the electric field manifested an inhibitory influence on the antibacterial capacity of the basil essential oil on four of the bacterial strains while its effect on *S. enteritidis* was slightly increased. There were no modifications on the antifungal capacity.

The highest antibacterial effects of basil essential oil 5 µL were on *S. aureus* and *E. coli*, yet they reached only 32, 95% and 29, 17% of the inhibition value of the control antibiotics Amoxycillin-clavulanic acid 30 µg and Enrofloxacin 5 µg.

CONCLUSION

The partial results of our study revealed that basil EO had a mild inhibitory effect on the investigated bacteria strains, except for *P. aeruginosa* that proved to be resistant and *B. cereus* on which it showed no relevant inhibition. The basil EO samples that were exposed to the electric field for 20 minutes showed a similar effect, with values a bit lower than the untreated essential oil samples. *P. aeruginosa* showed resistance to its influence, while *B. cereus* and *L. monocytogenes* had no inhibition response.

Since the composition of basil essential oil is so complex and it varies according to the region, the climate, the seasonal change and the extraction methods, these results might vary from the ones revealed by other studies (Liu Q et. Al, 2017). Hence, further research is needed in order to have a more rigorous perspective on the antibacterial and antifungal effects of basil essential oil as well on the influence of the electric field on its activity.

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