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Obtaining and Characterization of Volatile Oils from Aromatic Plants

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***Abstract*—The aim of this study was to extract the volatile oils from some aromatic plants and to investigate their antimicrobial and antioxidant activities. The volatile oils were isolated from dried parts of plants by hydro distillation using a neo-Clevenger apparatus. The volatile oils of basil (*Ocimum basilicum*), thyme (*Thymus vulgaris*), fennel (*Foeniculum vulgare*), lovage (*Levisticum officinale*), marjoram (*Majorana hortensis*) and dill (*Anethum graveolens*) were tested in three different quantities against *Salmonella typhi, Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Candida albicans* by disk diffusion method. The antioxidant activity of the volatile oils was determined by DPPH free radical scavenging method. The highest percentage yield of extraction was obtained for basil essential oil (1.26%). Thyme essential oil exhibited the best antimicrobial activity. Thyme essential oil showed an inhibition zone diameter of 50 mm when 1.5 µl of essential oil were tested against *S. typhi, B. cereus, E. coli* and *C. albicans*. Marjoram essential oil provided antimicrobial activity against all tested microorganisms. The diameter of inhibition zone observed for 1.5 µl of marjoram volatile oil tested against *S. typhi* was 17 mm. Lovage, fennel and dill essential oils were active against some bacterial and fungal strains. Basil essential oil was the less active. Thyme volatile oil showed the best antioxidant activity (87.28%). It was followed by lovage (34.99%), basil (30.27%) and marjoram (18.30%) essential oils. Fennel volatile oil and dill volatile oil did not possess antioxidant properties. This study shows that essential oils extracted form aromatic plants can inhibit the growth of some pathogens. Some volatile oils also has antioxidant activity. Therefore, volatile oils could be investigated for their use in pharmaceutical and food products.**

Keywords—volatile oils, extraction, aromatic plants, antimicrobial activity, antioxidant activity

I. INTRODUCTION

Volatile oils are complex volatile mixtures produced by aromatic plants as secondary metabolites [1]. Essential oils contains often a variety of components, especially terpenes and their oxygenated derivatives called terpenoids. It also contains phenylpropanoids, nitrogen or sulphur [2]. Since ancient times, volatile oils are known for their antibacterial, antifungal and antioxidant effects [3]. Nowadays, many microorganisms have become resistant to antibiotics. Therefore, it is necessary to investigate other sources of antimicrobial agents. Recent studies are investigating volatile oils with antimicrobial properties to combat microbial resistance [4]. The mechanism of antimicrobial action of volatile oils is due to the phenolic compounds. The volatile oils are hydrophobic and they can interact with the microbial cell membrane. This results in increased bacterial membrane permeability, disturb of cell structure and disturb homeostasis [5].

The oxidative stress is an important process that take place in the cells. It produces aging and degenerative diseases like cancer, cardiovascular diseases, multiple sclerosis, Parkinson's disease, autoimmune diseases and dementia [6, 7]. The antioxidants are substances that antagonize the action of free radicals [8]. Synthetic antioxidants such as butylhydroxytoluene (BHT) or butylhydroxyanisole (BHA) have been reported to have a carcinogenic effect. Natural antioxidants are highly studied due to their ability to protect the body from the harmful action of oxidative stress [6].

The aim of this paper was to extract the volatile oils from six aromatic plants (*Ocimum basilicum L.*, *Thymus vulgaris L.*, *Foeniculum vulgare Mill.*, *Levisticum officinale Koch.*, *Majorana hortensis Moench* and *Anethum graveolens L.*) and to investigate their antimicrobial and antioxidant activities. The antimicrobial activity was tested against six pathogens that frequently produce foodborne: *Salmonella typhi*, *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*.

II. EXPERIMENTAL

Plant materials

Dried aerial parts of *Ocimum basilicum L.* (basil), *Thymus vulgaris L.* (thyme), *Levisticum officinale Koch.* (lovage), *Majorana hortensis Moench* (marjoram) and dried seeds of *Foeniculum vulgare Mill.* (fennel) and *Anethum graveolens L.* (dill) were used for extraction the volatile oils. Except thyme aerial parts, all plant materials were purchased from a local shop. Thyme aerial parts were collected from Sibiu County, Romania and they were dried in the shade.

Volatile oils extraction

The volatile oils were extracted from plant materials by hydrodistillation using a modified neo-Clevenger apparatus for approximately 5 hours. After extraction the volatile oils were dried over anhydrous sodium sulfate and stored at 4^{O}C

in sealed vials until analysis [9]. The process of hydrodistillation was previously described by other researchers [10, 11].

The yield of extraction (η) was calculated by the following formula:

$$\eta = \frac{\text{volume of volatile oil (mL)}}{\text{weight of dried plant (g)}} * 100 \qquad (1)$$

Bacterial strains

In this study, four gram-positive bacteria (*Salmonella typhi*ATCC 1408, *Bacillus cereus*ATCC 12600, *Bacillus subtilis*ATCC 6051 and *Staphylococcus aureus*ATCC 12600), a gram-negative bacteria (*Escherichia coli*ATCC 11775) and a fungal strain (*Candida albicans*ATCC 10231) were tested. In general, these pathogens contaminate food and they are responsible for food poisoning [5].

Antimicrobial activity

The antimicrobial activity was performed by Kirby-Bauer disk diffusion method [4]. All volatile oils were tested against *S. typhi*, *B. cereus*, *B. substilis*, *Staph. aureus*, *E. coli* and *C. albicans*. The pathogens were activated on Nutrient broth for 48 h, then cultivated in Petri dishes with Muller Hinton II Agar as substrate. The antimicrobial activity was tested with three different volatile oil quantities: 0.5 µl, 1 µl, respectively 1.5 µl of volatile oils were added to a sterile paper disk placed in the middle of the Petri dishes containing the pathogen and the solidified substrate. Plates were incubated at 35^{o}C for 48 h. After 2 days, the diameter of the inhibition zone was measured with a ruler. All tests were performed in triplicate and the results show the average of the values.

Antioxidant activity

The antioxidant activity was determined by DPPH assay. The authors used a method similar with the method described by Tylkowski et al [12], with some modifications. A standard solution of 25 µg/ml DPPH in methanol was prepared. Seven solutions of DPPH with the concentration between 0.25 µg/ml and 2.5 µg/ml were prepared from the DPPH standard solution. The absorbance of the solutions was measured at 515 nm with a UV-VIS spectrophotometer (CECIL 1021), resulting the calibration curve. The equation of the calibration curve was y = 0.0127x + 0.0036, where x is the concentration of DPPH solution in µg/ml and y is the absorbance measured at 515 nm.

30 µl of volatile oil were added over 970 µl of DPPH 25 µg/ml standard solution. The absorbance of the samples was determined with CECIL 1021 spectrophotometer.

The concentration of DPPH in the samples was calculated with the following formula:

$$C\ (\mu g/ml) = \frac{A - 0.0036}{0.0127} \qquad (2)$$

Where:

C – the concentration of DPPH solution, in µg/ml;

A – the absorbance of the sample;

0.0036 – the intercept of the curve;

0.0127 – the slope of the curve.

The antioxidant activity was calculated by the following equation:

$$AA\ (\%) = \frac{C_0 - C_1}{C_0} * 100 \qquad (3)$$

Where:

C_0 – the DPPH standard solution concentration

C_1 - the DPPH concentration after it reacts with the antioxidant

III. RESULTS AND DISCUSSION

The percentage yields of extraction are 1.26% for *Ocimum basilicum L.* volatile oil, 1.18% for *Foeniculum vulgare Mill.* volatile oil, 0.96% for *Thymus vulgaris L.* volatile oil, 0.73% for *Anethum graveolens L.* volatile oil, 0.56% for *Levisticum officinale Koch.* volatile oil and 0.3% for *Majorana hortensis Moench* volatile oil.

Fig. 1-6 present the results obtained after antimicrobial activity analyses.

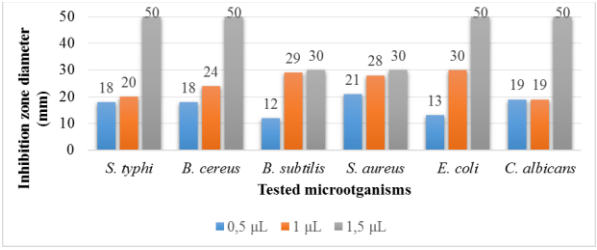

Fig. 1. Antimicrobial activity of *Thymus vulgaris L.* volatile oil

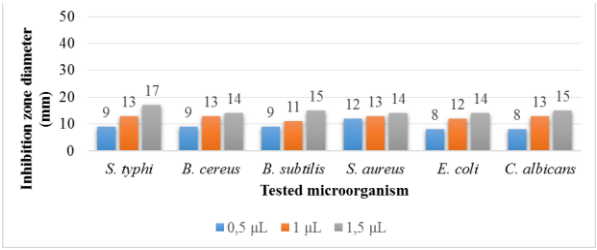

Fig. 2. Antimicrobial activity of *Majorana hortensis Moench* volatile oil

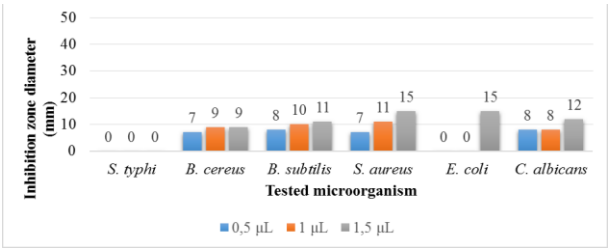

Fig. 3. Antimicrobial activity of *Levisticum officinale Koch.* volatile oil

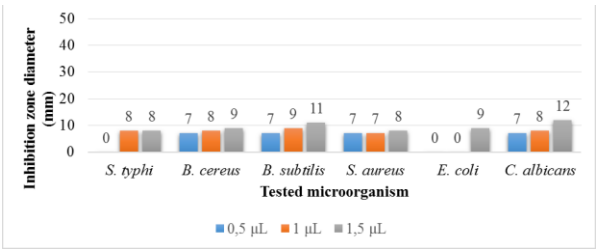

Fig. 4. Antimicrobial activity of *Foeniculum vulgare Mill.* volatile oil

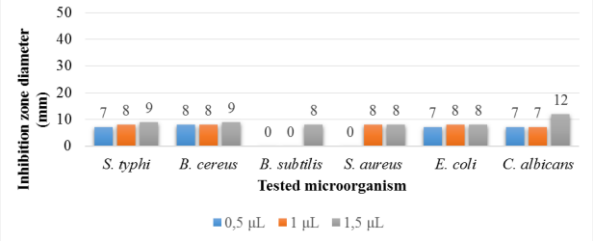

Fig. 5. Antimicrobial activity of *Anethum graveolens L.* volatile oil

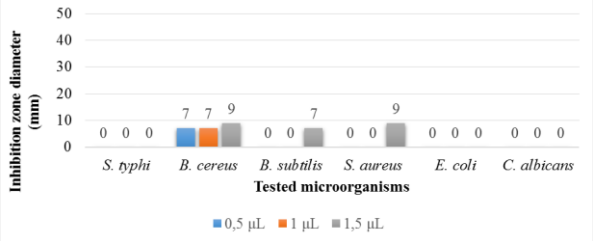

Fig. 6. Antimicrobial activity of *Ocimum basilicum L.* volatile oil

DPPH free radical scavenging method results are summarized in Table I.

The volatile oils extracted from fennel seeds (*Foeniculum vulgare Mill.*) and dill seeds (*Anethum graveolens L.*) did not show antioxidant activity.

Compared with another studies, the yields of extraction for basil and lovage volatile oils in the present study were higher than the yields reported by Semeniuc et al, but in our research the yield of extraction for thyme volatile oil was smaller than the yield reported by Semeniuc et al [13]. Fennel volatile oil percentage yield in the present study was close to the yield determined by Singh et al [14]. Busatta et al reported a yield of 1.2% for marjoram volatile oil, a higher percentage than in our study [15]. The percentage yield of dill volatile oil reported by Jianu et al was 0.92% [16], close to the yield determined in the present study. The differences in the percentage yields are due to variation in quantity, which is correlated with growing conditions (climate, soil constituents), the part of the plant used for extraction, the stage of ripening process.

In our study, thyme volatile oil showed the highest antimicrobial activity. It was active against all tested pathogens: *S. typhi*, *B. cereus*, *B. subtilis*, *Staph. aureus*, *E. coli* and *Candida albicans*. Also, marjoram volatile oil was active against all tested microorganisms, but the diameters of the inhibition zones were smaller compared with the diameter measured for thyme volatile oil. According to our results, some microorganisms were resistant to lovage, fennel and dill volatile oils. Basil volatile oil presented the weakest antimicrobial effect.

TABLE I. ANTIOXIDANT ACTIVITY OF VOLATILE OILS

Volatile oil	AA (%)
Thymus vulgaris L. volatile oil	87.28
Levisticum officinale Koch. volatile oil	34.99
Ocimum basilicum L. volatile oil	30.27
Majorana hortensis Moench volatile oil	18.30

There are several studies in the literature which present the antimicrobial activity of volatile oils, but the amount of volatile oil used was bigger than in our study. In a study conducted by Borugă et al, they also reported that thyme volatile oil has an antimicrobial effect against *Staph. aureus*, *S. typhi*, *E. coli* and *Candida albicans* [17]. Semeniuc et al reported that thyme volatile oil has strong antimicrobial effect against *Escherichia coli*, moderate effect against *Salmonella typhi* and *Bacillus cereus* and mild antimicrobial effect against *Staphylococcus aureus*. They also reported a mild antibacterial effect of basil volatile oil against *B. cereus*. In contrast with our study, Semeniuc et al reported that basil volatile oil has a mild antimicrobial effect against *E. coli* and *S. typhimurium* [13].

DPPH free radical scavenging method shows that thyme volatile oil has the highest antioxidant activity (AA=87.28%). Lovage volatile oil has lower antioxidant activity (AA=34.99%) than thyme volatile oil, but higher than basil volatile oil (AA=30.27%). Marjoram volatile oil presents the weakest antioxidant activity (AA=18.30%). Fennel volatile oil and dill volatile oil have no antioxidant activity.

In the literature, there are other studies that confirm the antioxidant capacity of essential oils. DPPH radical scavenging activity is not the only method to determine the antioxidant activity. Other researchers tested antioxidant activity of volatile oils by ABTS Radical Cation Scavenging Activity, β-Carotene-Linoleic Acid Bleaching Assay, Chelating Effect on Ferrous Ions and Reducing Power Assay [1, 6, 18].

IV. CONCLUSION

In conclusion, the percentage yield of extraction is low due to the low content of volatile oil in aromatic plants. For this reason, the volatile oils are so expensive.

This study shows that volatile oils are effective against some pathogens that produce food poisoning. Thyme volatile oil shows the best antimicrobial activity. It is followed by marjoram, lovage, fennel, dill and basil volatile oils. The data obtained are important for further investigations regarding the treatment of infectious diseases. In future studies we intend to determine the antimicrobial action of these oils at higher concentration and to determine the minimum inhibitory concentrations and the minimum bactericidal concentration.

In addition, the research shows that the tested volatile oils possess antioxidant activity, except dill and fennel volatile oils. These findings confirm that some essential oils can be used to combat the oxidation in different systems. A suggestion for future studies is to test the antioxidant activity with other free radicals.

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