ANTISEPTIC PROPERTIES OF ESENTIAL OILS AND SOME ASPECTS REGARDING THEYR EXTRACTION FROM SOME SPECIES OF MEDICINAL PLANTS

Gabriela Valentina CIOBOTARU[1]; Ilinca Merima IMBREA[1]; Dana COPOLOVICI[2] V. DUMITRAȘCU[3]; Georgeta POP[1].

[1] Banat's University of Agricultural Sciences and Veterinary Medcine „King Michael I of Romania" from Timisoara, **Romania;**
[2]"Aurel Vlaicu" University, **Arad, Romania;**
[3]University of Medicine and Pharmacy „Victor Babeș" Timișoara, Romania.

Corresponding author: vaciobotaru@gmail.com

Abstract: *Consumers are demanding more and more the use of natural foods with a low or zero quantity of chemical additives. Essential oils found in medicinal plants have presented interest in food industries because of their antibacterial and antioxidant properties. The aim of this study is to test the antimicrobial activity of essential oils extracted from 4 species of medicinal plants: Hyssopus officinalis, Rosmarinus officinalis, Salvia officinalis and Thymus vulgaris against the bacterial strains: Staphylococcus epidermidis, Staphylococcus aureus and Escherichia coli. Fluconazole 25* µg *was used as negative control and Chloramphenicol 30* µg *was used as positive control. It is well known that the quantities and concentrations of the active compounds of most esential oils are strongly dependent on the climatical conditions of the medicinal plants' origin, time of harvest and processing methods, we adressed as well on some aspects of the extraction process itself, some morfological and pedo-climatical conditions provided by the Young Naturalist Resort from Timisoara where the plants were grown and where this study took place.*

Keywords*: Hyssopus officinalis, Rosmarinus officinalis, Salvia officinalis, Thymus vulgaris, essential oil, antiseptic, extraction*

INTRODUCTION

Nowadays, consumers demand the use of natural food products, with low or even no chemical additives. Essential oils found in medicinal plants have presented interest in the food industry because of their antibacterial and antioxidant properties, making the study of the inhibitory effect of those oils in microorganisms an alternative to reduce the use of chemical additives in foods. Climate and soil conditions in the western region of the country, Banat, favored the development of a diverse wealth and impressive variability. Regarding the number and the importance of species with pharmaceutical interest, *Lamiaceae* family, appears as one of the most important, providing an immense field for study and research. *Lamiaceae* or *Labiatae* family is an important dicotyledonous family comprising about 6.000 species and over 210 genres. Their importance is due primarily to therapeutic essential oils, with antibiotic action and also to odor and bitter substances recommended in biliary disease (PALICI, 1997).

Hyssopus officinalis L. has been traditionally used for its antiseptic properties in treatment of infectious disorders (ABU-DARWISH, 2013). We have screened the antimicrobial activity of the aerial parts of *Hyssopus officinalis* L. essential oil against different microorganisms. It is clearly that the essential oil of hyssop possesses compounds with antimicrobial properties, which could be used as antimicrobial agents in new drugs for therapy against human infectious diseases (SHAROPOV, 2012), (MAHBOUBI, 2013). The harvesting

process should begin before the blooming phase, when the volatile oil content is at it's peak. (MUNTEAN, 2007).

Salvia officinalis L.(sage, garden sage, or common sage) is a perennial, evergreen subshrub, with grayish leaves, woody stems and blue to purplish flowers. It is native to the Mediterranean region but currently it is cultivated in various countries around the world. M. S. Abu-Darwish & al. proved that *Salvia officinalis* L. EO has antifungal activity against dermatophyte strains.

The antimicrobial activity of *Hyssopus officinalis* L. essential oil was tested against three bacterial strains: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and *Staphylococcus epidermidis* (ATCC 14990) using the agar disk-diffusion method and chloramphenicol respectively fluconazole as reference. The results obtained in this study indicate that the *Hyssopus officinalis* L. essential oil has a lower effect as chloramphenicol over all three bacterial strains. *Staphylococcus aureus* had a higher sensitivity to the *Hyssopus officinalis* L. essential oil, creating a 20mm area of inhibition, but lower than chloramphenicol 30 µg, which created an area of inhibition that measured 30 mm. Fluconazole 25 µg only inhibited *Escherichia coli* creating a 14 mm inhibition area, which is similar to the inhibition area that was created by the *Hyssopus officinalis* L. essential oil to the same bacteria (14 mm). *Staphylococcus epidermidis* had the following inhibition measurements: 15 mm for the *Hyssopus officinalis* L. essential oil and 30 mm for chloramphenicol 30 µg. The essential oil of hyssop is less effective for the three bacterial strains studied compared with chloramphenicol, but shown stronger or equal effects, compared to fluconazole.

MATERIAL AND METHODS

The antimicrobial activity of the extracts was determined through the disk diffusion method, on Mueller Hinton agar, using impregnated paper disks. Chloramphenicol and Fluconazole were used as reference for the antibiotic activity. Identification of the species was confirmed by the department of Aromatic plants from USAMVB Timisoara and a voucher specimen was preserved.

Isolation of essential oil.

Salvia officinalis L. was grown in a temperate climate zone in Timisoara, Romania ($21^0 13`$E longitude, $45^0 45`$ N latitude). The harvest took place in 11.07.2016 during the blooming period after a period of sunny days because sun light has a positive influence on the synthesis of the volatile oil.

Essential oil from 4 species of medicinal plants*: Hyssopus officinalis, Rosmarinus officinalis, Salvia officinalis and Thymus vulgaris* whole herba, was extracted by continuous steam distillation (Soxhlet extraction) using classic Clevenger equipment and tested against 3 bacterial strains: *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Escherichia coli*. Fluconazole 25 µg was used as negative control and Chloramphenicol 30 µg was used as positive control.The fresh herb was dried in a room with no sunlight access at a temperature between 20 and 22°C. The essentail oil was obtained through hydrodistillation using a volatile oil distilling Clevenger equipment. The essential oil was kept at +4°C until analysis.

Gas chromatography/mass spectrometry identification.

The chemical composition of the essential oil was determined using gas chromatography/mass spectrometry (GC/MS) analysis. Agilent Technology 7820A (AGILENT Scientific, USA) coupled with mass spectrometer MSD 5975 and equiped with a capillary column DB 5: (30 m X 250 µm X 0.25 µm, Agilent, USA) was used. The carrier gas was helium with a mass flow of 1 $mL \cdot min^{-1}$. In order to separate the compounds and the following GC oven program was used: 40 °C for 1 min, 5 °C min^{-1} to 210 °C for 5 min. The injector and

ion source temperatures were 250 and 150°C, respectively. The injection volume was 1 µL with a split ratio 1:20. The NIST spectra library has been used to identify the volatile compounds.

RESULTS AND DISCUSSIONS

Among several essential oils that may be useful as antimicrobial agents, *Staphylococcus aureus* ATCC 25923 (inhibition area in mm) inocul 1,5x10^8 CFU/mL, *Hyssopus officinalis* 20mm, *Rosmarinus officinalis* 25mm, *Salvia officinalis* 20mm *anThymus vulgaris* 30, compared to Chloramphenicol 30µg and Fluconazol 25 µg.

Essential oil may have the greatest potential for use in industrial applications. The main scope of this study is testing the antibacterial effects of *Hyssopus officinalis* 14 mm, *Rosmarinus officinalis* 20 mm, *Salvia officinalis* 0 *and Thymus vulgaris* 15 mm on four different bacterial strains *Escherichia coli ATCC 25922* (inhibition area in mm) compared to Chloramphenicol 30µg and Fluconazole 25 µg.

Table 1

Inhibition of 4 species medicinal plants essential oil compared to the reference antibiotics

Reference strain/Name of the species	Chloram-phenicol 30 µg	Flucona-zole 25 µg	EO *Hyssopus officinalis*	*EO Rosmari-nus officinalis*	*EO Salvia officina-lis*	*EO Thymus vulgaris*
S.aureus ATCC 25923 (inhibition area in mm) inocul 1,5x10^8 CFU/mL	30mm	0	20	25 mm	20 mm	30 mm
E.coli ATCC 25922 (inhibition area in mm)	30 mm	15 mm	14 mm	20 mm	0	15 mm
Staphylococcus epidermidis ATCC 14990 (inhibition area in mm)	30 mm	0	15	0	15 mm	30 mm

Among several essential oils that may be useful as antimicrobial agents against *Staphylococcus epidermidis* ATCC 14990 (inhibition area in mm), *Hyssopus officinalis* 15 mm, *Rosmarinus officinalis* 0 mm, *Salvia officinalis* 15 mm and *Thymus vulgaris* 30 mm, compared to Chloramphenicol 30µg.

Among the analyzed oils the main chemotypes have been identified: in the composition of volatile oil extracted from the species *Hyssopus officinalis*, the chemotype with the highest weight of 24.9% is sabinene, followed by two compounds with close values, limonine (18.9%) and b-pinene (18.0%). Significant amounts also include 13.7% linalool and 10.1% a-pinene.

The other six compounds, identified in reduced amounts, with values ranging from 1.1% p-mentha-1,4 (8) diene and 4.1% a-phellandrene, are shown in Figure 1.

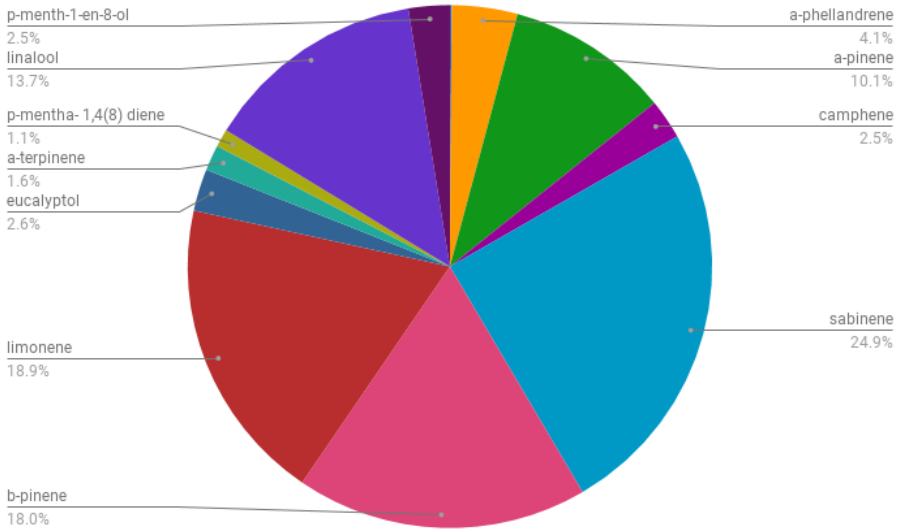

Figure 1. *Hyssopus officinalis* percentage of main compounds

Among the analyzed species, in *Hyssopus officinalis* and *Rosmarinus officinalis* we find compounds common to both such as: a-phellandrene; a-pinene; camphene; sabinene; b-pinene; limonene; eucalyptol; a-terpinene; p-mentha- 1,4(8) diene; linalool and p-menth-1-en-8-ol.

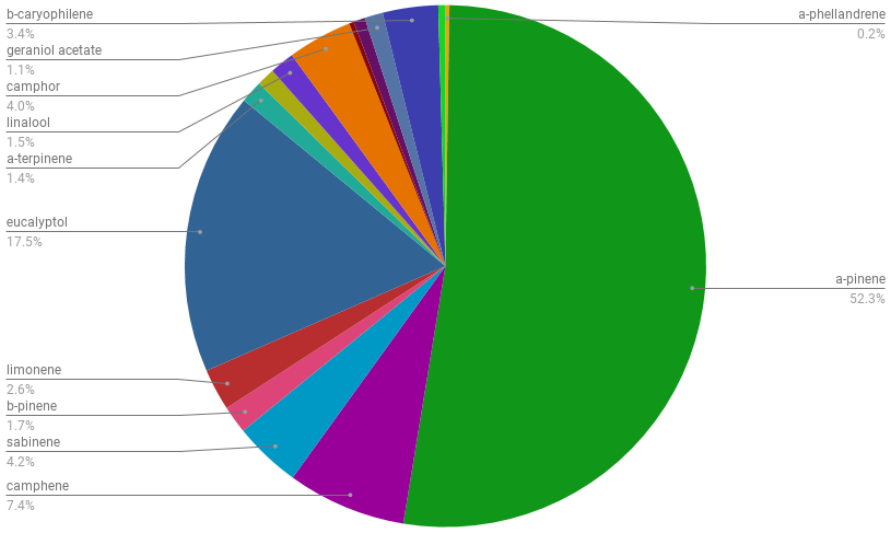

Figure 2. *Rosmarinus officinalis* percentage of main compounds

Figure 3 shows the content in volatile compounds as it has been determined from *Salvia officinalis* oil, the prevalent chemotype being 36.7% eucalyptol. Similar values have

been also found in the analyzed compounds: 17.5% caphene chemotype, *a-pinene* 14, 6% and 10.4% *geranyl acetate*.

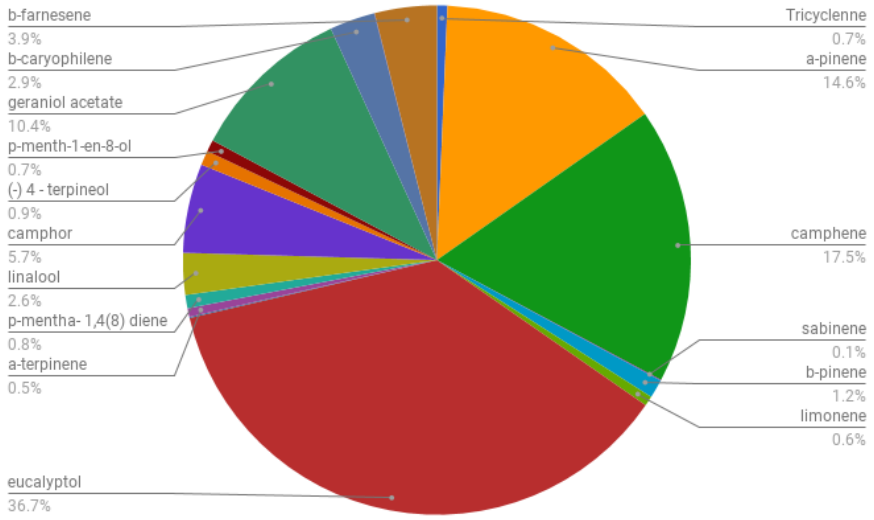

Figure 3. *Salvia officinalis* percentage of main compounds

The smallest values of 1% were recorded as follows: 0.1% sabinene, 0.5% a-terpinene, 0.7% p-menth-1-en-8-ol, 0,7% tricyclenne and 0.9% 4-terpineol.

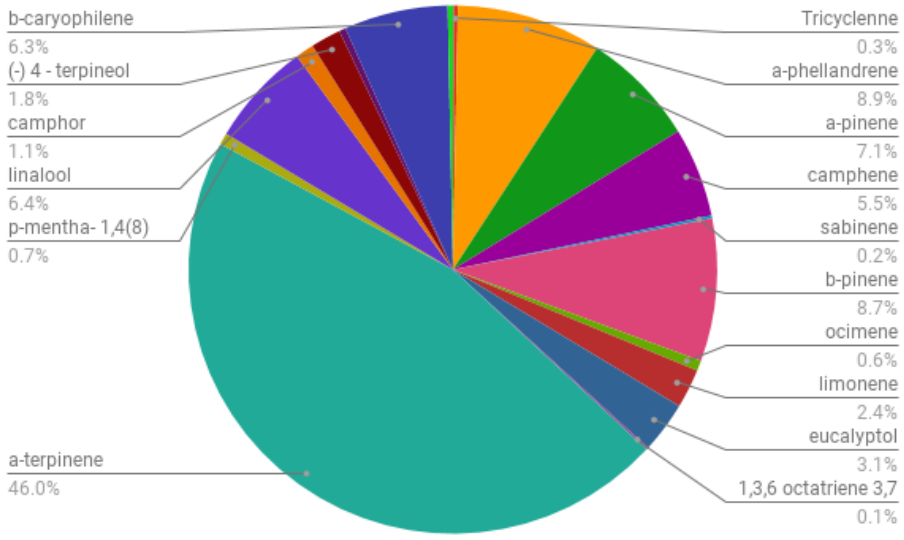

Figure 4. *Thymus vulgaris* percentage of main compounds

Figure 4 presents the chemical composition of oil extracted from *Thymus vulgaris*, considering the chemical compound a-terpinene found in the highest percentage of 46% of the total compounds.

As far as the *Thymus vulgaris* oil is concerned the following compounds, with the highest representation, were identified: 8.9% a-phellandrene, 8.7% b-pinene, 7.1% a-pinene, 6.4% linalool 6.3 % b-caryophyllene. Five chemotypes with the lowest values of less than 1% have been identified in *Thymus vulgaris*.

CONCLUSIONS

In this in vitro study, we have shown that essential oil from 4 species of medicinal plants can be very efficient as an antibacterial agent. It has shown stronger and or equal effect as the common used antibacterial agents.

The content of volatile compounds, or the dominant chemotype, differs depending on the variety, the place of cultivation and the time of harvesting.

The volatile oils have showed considerable inhibitory effects against the tested reference strains, while their major components have demonstrated various degrees of growth inhibition.

In view of above facts, the medicinal plants belonging to *Lamiaceae* family cultivated in western part of Romania show great promise as a dietary and therapeutic source involved in human health. These data are important for nutritional and pharmacological assessment, medicinal plant labeling and consumer education.

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