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Original Article

Chemical composition and antimicrobial activities of volatile oils in some *Lamiaceae* species

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Abstract

The chemical composition of Thymus pannonicus L. and Perovskia atriplicifolia Benth. volatile oils and their antimicrobial activity were studied. Volatile oils of the aerial parts of the plant were obtained by hidrodistilation and analyzed using GC-MS. Antimicrobial activity was evaluated for the volatile oils and for their main components. Our study indicates that the volatile oils of these species have considerable antimicrobial activity deserving further investigation for clinical application.

Key-words: *volatile oil, antimicrobial activity, Lamiaceae, Thymus, Perovskia.*

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Introduction

The *Lamiaceae* plant family is one of the largest families among the dicotyledons, being composed of more than 240 genera, many species belonging to the family being highly aromatic, due to the presence of external glandular structures that produce volatile oil (4). This oil is important in pesticide, pharmaceutical, flavouring, perfumery, fragrance and cosmetic industries (10). *Thymus pannonicus* L. is a perennial herbaceous plant, distributed in central and eastern Europe as well as in Russia, over open dry grasslands and rocks. In Romania, this species presents vigorous branched stems, covered with hairs with the same length of the axis diameter (2). The dried herb of *Th. pannonicus* L. is used to make tasty and refreshing herbal tea drinks, due to its peculiar and pleasant lemon-like scent. Fresh leaves are used for aromatization of homemade jams, candies and similar confectionery. It is also being used with positive results for coughs and other respiratory complaints, as well as some cases of gastrointestinal disorders. *Perovskia atriplicifolia* Benth. originates and grows on the rocky areas in Afganistan and Pakistan and it is known for its febrifuge properties, and beside its medical use in reducing fever, is also used in culinary and decorative purposes. In Europe it is mainly used as a decorative plant (12).

Material and Methods

The aerial parts of *Thymus pannonicus* L. and *Perovskia atriplicifolia* Benth. were collected during the flowering period from Suceava district and Iaşi district. The volatile oils have been extracted using a Clevenger type apparatus according to the European Pharmacopoeia standards, in the Laboratory of Plant Physiology, Faculty of Biology, "Alexandru Ion Cuza" University from Iaşi. The separation and the identification of the components have been carried out using GC-MS (gas chromatography coupled with mass spectrometry) at the Faculty of Horticulture, U.S.A.M.V. Bucureşti.

To test the antimicrobial effect of the volatile oils extracted from *Ocimum basilicum* and *Perovskia atriplicifolia*, the diffusimetric method on nutritive culture medium has been used (9), using stainless steal cylinders. To stimulate the diffusion of the tested oils on the substrate, in L the samples and in control, Tween 80, 0,5 % v/v has been added (13).

Results and Discussion

Chemical composition of the volatile oils

Concerning the investigation of volatile oil in *Thymus pannonicus* and *Perovskia atriplicifolia* the main characteristic constituents were identified-these constituents having specific properties in each sample. GC-MS analysis of volatile oils resulted in the identification of 25 compounds for *Th. pannonicus* and 28

compounds for *Perovskia atriplicifolia* (Table 1).

The main constituents of *Thymus pannonicus* volatile oil are: α-terpinyl acetate (48,83%), germacrene D (12,12%), cariophyllene oxide (6,35%) and mircene (4,73%) (fig. 1).

Germacrene D is a hydrocarbon in the sesquiterpene class and it can be used as a pesticide and pheromone (http://sun.ars-grin.). The cariophyllene oxide has anti-inflammatory proprieties (11) and it can also be used as an insecticide and fungicide (1). Mircene is a monoterpene utilized in perfumery and also in medical purposes as an analgesic, as a fungicide and as an antibacterial substance (6). Concerning the chemical composition of the volatile oil of *Perovskia atriplicifolia*, the main constituents are: limonene (18%), cymene (18%), borneole (15%), cis-β-cimene (8%) and γ-terpinene (7%) (fig. 2).

Limonene, the main constituent of the volatile oil of *Perovskia atriplicifolia* can be used to Fight virosis, as well as an expectorant, pesticide, as a sedative (15). Cymene, one of *Th. pulegioides* oil constituents with the highest amount, is used in pesticides, laxatives and sedatives. Concerning the chemical composition of the volatile oil, in a survey of available literature, only few publications relevant for the chemical composition of *Th. pannonicus* were found, suggesting that a substantial lack of information in this field still exists. A greater interest in this essential oil was expected as this species is neither endemic, nor endangered across the whole area of its distribution. According to Karuza-Stojaković *et al.*, (6) the principal constituents of *Th. pannonicus* essential oil from southern parts of Vojvodina province are terpinyl acetate, terpinen-4-ol, thymol, carvacrol and geranyl acetate (listed in order of descending quantity). Recent comprehensive studies of chemical variability in hydrodistilled volatile oils of different wild growing and cultivated populations of *Th. pannonicus* from Hungary, as well as supercritical fluid extracts of various *Lamiaceae* species, confirmed that high concentrations of both thymol and *p*-cymene are the main chemosystematic attributes of *Th. pannonicus* essential oil. The chemical composition of volatile oils of *Thymus* species present a high variability and diversity- at least 20 different chemotypes have been established thus far [14]. The production of phenolic compounds is higher in warmer and drier climatic areas, non-phenolic compounds usuLy accumulate in higher quantities in colder and damper areas (7). The volatile oil of *Perovskia atriplicifolia* Benth. species has recently been studied (5,3), in Romania, such studies have not been carried out due to the fact that the species has recently been introduced and cultivated, being strictly used as an ornamental plant.

Antimicrobial activity of volatile oils

The antibacterial activity of volatile oils of the two analyzed species has been carried out using *Staphylococcus aures* ATCC-6538 and *Escherichia coli* ATCC-10536 test strains. The antibacterial activity has been determined measuring the diameter of the inhibition area (in mm) around the cylinders containing the tested concentration (1:1, 1:5, 1:10) that were applied on the nutritive culture medium previously inoculated with the test microorganism. The results were continually compared to the control sample (fig. 5-6). The analysis of the volatile oil extracted from *Thymus pannonicus* showed an antimicrobial effect on the Gram positive bacteria - *Staphylococcus aureus*, and also on the Gram negative bacteria - *Escherichia coli*, for L dilutions (fig. 3, 4). The antimicrobial analysis of the *Perovskia atriplicifolia* extracted oil showed that it has an antimicrobial effect only on the Gram positive bacteria - *Staphylococcus aureus* (fig. 7, 8). If we correlate these data with those regarding the chemical composition of the *Thymus pannonicus* volatile oil, we can conclude that its strong antibacterial activity shown on the two test strains is due to the high concentration of α-terpinyle acetate. The antimicrobial potential of *Perovskia atriplicifolia* volatile oil that could result from the presence of some compounds exhibiting inhibitory effect on the growth of the bacteria *Staphylococcus aureus* is demonstrated in the literature of specialty: α-terpineole (2) and charvacrole (2,8).

Conclusion

In conclusion, we can affirm that *Thymus pannonicus* volatile oil has a strong antibacterial effect on both Gram positive bacteria - *Staphylococcus aures*, and on the Gram negative bacteria - *Escherichia coli*. Bacterial growth and development inhibition could be a result of the high quantity of α-terpinyle acetate (48,83%) in the oil composition. *Perovskia atriplicifolia* volatile oil has an antibacterial effect on the species *Staphylococcus aureus*. No effect has been noticed on the tested Gram negative bacteria (*Escherichia coli*). The inhibitory effect induced on the growth of the test strain could be due to the presence of α-terpineole and charvacrole in the composition of the tested volatile oil.

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Table 1. Chemical composition of *Thymus pannonicus* and *Perovskia atriplicifolia* volatile oils.

Compounds	*Perovskia atriplicifolia* (%)
Triciclene	0,14
α-Thujene	1,30
α-Pinene	4,00
Camfene	4,50
Sabinene	0,30
β-Pinene	3,60
Mircene	1,09
α-Felandrene	-
α-Terpinene	0,85
Cymene	17,93
Limonene	17,75
cis-β-Ocimene	8,37
γ-Terpinene	7,15
α-Terpinolene	0,30
Linalool	0,75
Thujone	0,20
cis-Sabinole	0,30
Borneole	14,67
Terpinen-4-ol	0,37
α-Terpineole	0,37
p-Timole	0,30
Bornyl acetate	4,02
Charvacrole	0,16
Terpinyl acetate	1,95
β-Caryophyillene	3,90
α-Caryophyillene	3,60
Caryophyllene oxide	1,05
Epiglobulole	0,45
Globulole	0,30

Compounds	*Thymus pannonicus (%)*
Sabinene	0,34
Mircene	4,73
Octinyl acetate	1,16
Limonene	2,69
cis-ß-Ocimene	2,61
p-Cymene	0,59
α - terpineole	0,44
Cis-Geraniol	0,30
ß - Citral	0,89
α - Citral	1,20
Trans-Geraniol	1,46
α -Terpinyl acetate	48,83
Geranyl acetate	0,5
Neryle acetate	2,21
α - Burbonene	0,98
ß-Cariophyllene	1,32
GermacreneD	12,12
Elemene	0,58
ß-Bisabolene	1,14
δ-Cadinene	0,69
Spatulenol	2,29
Cariophyllene oxide	6,35
α – Cadinol	1,09
Izoaromodendren epoxid	0,53
Trans-Farnesol	1,45

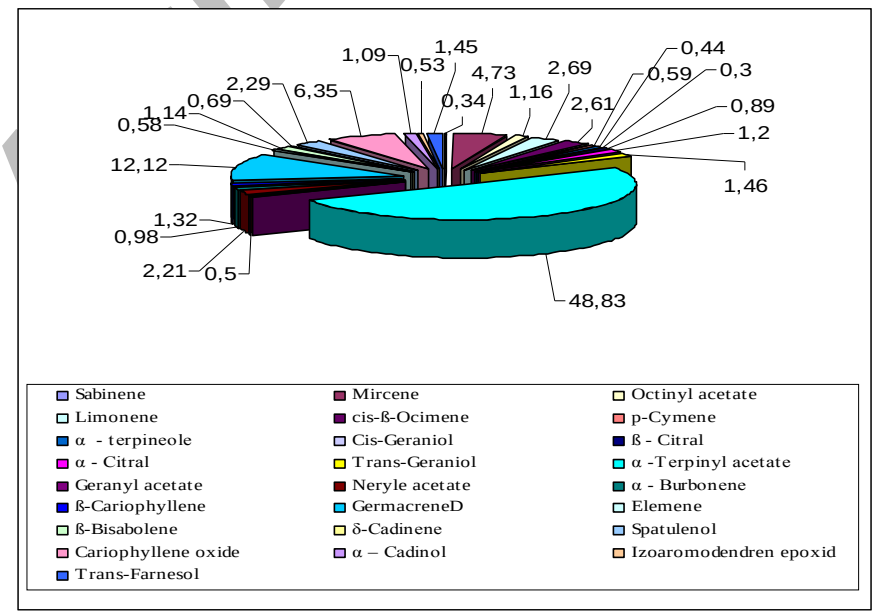

Fig 1. Components of volatile oil of Thymus pannonicus L.

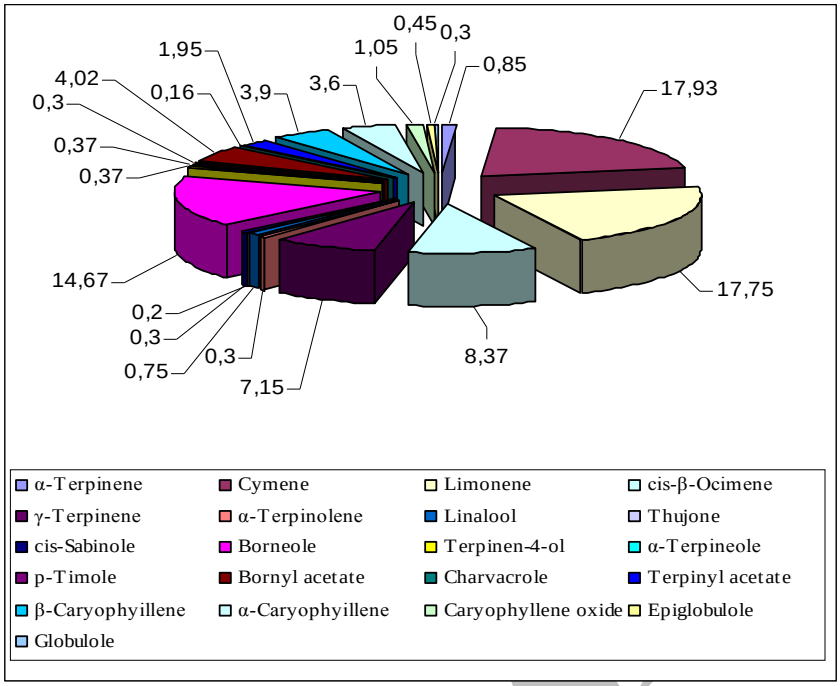

Fig2. Components of the volatile oil of Perovskia atriplicifolia Benth.

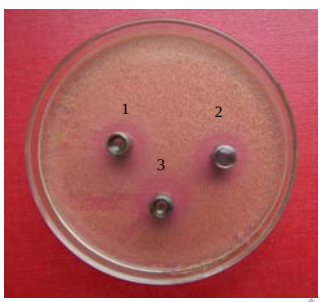

Fig. 3. Antibacterial activity of *Th. pannonicus* volatile oil on *S.aureus* strain.(1, 2, 3 different concentration).

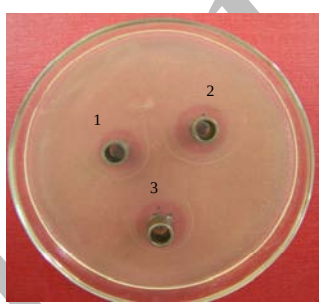

Fig. 4. Antibacterial activity of *Th. pannonicus* volatile oil on *E. coli* strain .(1, 2, 3 different concentration).

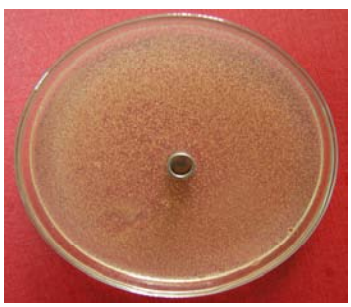

Fig. 5. Control sample for *S. aureus*.

Fig. 6. Control sample for *E. coli*.

Fig.7. Antibacterial activity of *Perovskia atriplicifolia* volatile oil on *S.aureus* strain (1, 2, 3 different concentration, M – control sample.)

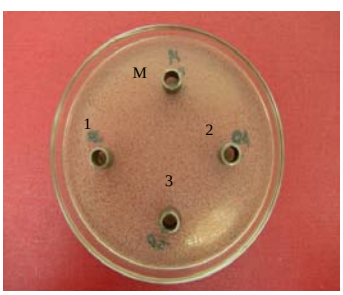

Fig.8. Antibacterial activity of *Perovskia atriplicifolia* volatile oil on *E. coli* strain (1, 2, 3 different concentration, M – control sample.)