 Medicamentul Veterinar / Veterinary Drug
Vol. 17(1):100-105; May-June 2023

Evaluation of the chemical composition and antimicrobial effect of Thuja essential oil

Evaluarea compoziției chimice și a efectului antimicrobian al uleiului esențial de Thuja

Eugenia Dumitrescu, Mihai Folescu, Cristina T. Romeo
Faculty of Veterinary Medicine Timișoara

eugeniadumitrescu@usvr.ro

Cuvinte cheie: ulei esențial de Thuja, chimis, antimicrobian
Keywords: Thuja essential oil, chemical, antimicrobial

Abstract

Starting from the consideration that the pharmacological and therapeutic properties of an essential oil are given by their chemical components, components that differ depending on the origin, soil, temperature, humidity, etc., we set out to determine the content of volatile compounds in the oil essential thuja purchased from the trade and to test its antimicrobial effect. The objectives of the research were to identify volatile compounds from thuja essential oil by the GS-MS method and to test the antimicrobial effect on gram positive and gram negative species. The obtained results showed that Thuja essential oil has significant inhibitory effects against gram-positive and gram-negative bacteria, which are associated with clinical diseases; it is more effective against gram positives than gram negatives; ineffective against Salmonella typhimurium and the major components identified were: α-pinene, 3-carene, terpinolene, limonene, β-myrcene and camphene. The presence of these compounds shows that thuja essential oil has a great potential for use in many medical applications.

Rezumat

Plecând de la considerația că proprietățile farmacologice și terapeutice ale unui ulei esențial sunt date de componentele sale chimice, componente care diferă în funcție de origine, sol, temperatură, umiditate etc., ne-am propus să determinăm conținutul de compuși volatili din ulei esențial de Thuja achiziționat din comerț și pentru a-i testa efectul antimicrobian. Obiectivele cercetării au fost identificarea compușilor volatili din uleiul esențial de tuia prin metoda GS-MS și testarea efectului antimicrobian asupra speciilor Gram pozitive și Gram negative. Rezultatele obținute au relevat că uleiul esențial de Thuja are efecte inhibitoare semnificative împotriva bacteriilor Gram-pozitive și Gram-negative, asociate cu boli la animale; cu eficiență mai crescută împotriva germenilor Gram-pozitivi comparativ cu Gram-negativii, fiind ineficientă împotriva lui *Salmonella typhimurium*, iar componentele majore identificate au fost: α-pinenă, 3-carenă, terpinolen, limonen, β-mircen și camphene. Prezența acestor compuși arată că uleiul esențial de thuja are un mare potențial de utilizare în multe aplicații medicale.

1. Introduction

Thuja spp is a hermaphrodite conifer, belonging to the genus *Thuja*, subfamily *Thujoideae*, Family *Cupressaceae*. The popular name of this tree is the "tree of life" [12].

The genus *Thuja* includes 2 species that are native to North America (*Thuja occidentalis, Thuja plicata*) and 3 species that are native to Western Asia (*Thuja koraiensis*, *Thuja standishii*, *Thuja sutchuenensis*) [1].

The medicinal parts are the leaves, cones and stem, from which tinctures, extracts and essential oils can be obtained. In the 19th century, trees of this genus were used in the form of tincture or ointment to treat warts, warts, dermatophytoses and oral candidiasis. The indigenous people of Canada used *Thuja occidentalis* leaves to make a tea that they consumed to prevent scurvy. It has been shown to contain 50 mg of vitamin C in 100 g of product [9].

Over time, the antiviral, antioxidant, antimicrobial and antidiarrheal effects of *Thuja occidentalis* extracts have been demonstrated, extracts that have been used to treat bronchial catarrh, cystitis, psoriasis, uterine carcinomas and rheumatism. The essential oil obtained from these trees contains thujone, a substance that has been studied for its antagonistic effect on GABA receptors [10].

Currently, essential oils or tinctures are used, both of which have an antibacterial effect. Thuja extract has been shown to have an antibacterial effect, acting on both Gram-positive and Gram-negative bacteria [4].

It is more often used in the treatment of acute or chronic infections of the upper respiratory tract, as an adjunctive antibiotic in severe bacterial infections, such as bronchitis, angina, pharyngitis, otitis media and sinusitis.

The immunostimulatory and antiviral properties are based on increasing the proliferation of T lymphocytes and the production of interleukin-2.

From a chemical point of view, thuja contains a number of compounds: saponins, phenols, tannins, amines, mucilages, bitter principles, lactonic components, carotene, essential oils, triterpenes, steroids, reducing carbohydrates, coumarins, tannic acid, polysaccharides, proteins and minerals.

Among all these components, flavonoids and lignans are representative compounds for this group of trees. From the group of flavonoids, the following were identified: catechin, gallocatechin, mearusitrin, myricetin, procyanidin B-3, prodelphinidin, querectin, quercitrin and kaempferol, and among the bioflavonoids: bilobetin and amentoflavone [2].

Amentoflavone, a compound present in considerable amount in *T. occidentalis* shows high antifungal activity against highly pathogenic fungi and antiviral properties against respiratory syncytial virus. Also, lignans are polyphenols that over time have been associated with strong antioxidant, antiviral, antibacterial, insecticidal and nematocidal activity [13].

Thuja occidentalis possesses a series of pharmacological properties, which are exerted by active substances such as: α-pinine, d-α-thujone, 1-frencone, 1-broneol acetic, andisovaleric acid, terpineol, sabiene, camphene, valerianic camphor acid, occidol-β -sitosserol, quercetin rhodoxanthin, tannins, mucilages and vitamin C [3, 7].

Also, hepatoprotective activity has been demonstrated through the protection it provides to the liver in acute or chronic liver failure, through flavonoids, tannins and polysaccharides [5].

Phenolic compounds act protectively on the gastric mucosa in lesions caused by aspirin, stress, alcohol or hydrochloric acid [6].

2. Materials and methods

2.1. Thuja essential oil

Thuja essential oil, commercially available oil, was used in the study.

According to the manufacturer's specifications, thuja oil was obtained by steam distillation of thuja branches and bark. It has a woody, warm, earthy aroma.

Figure 1. Thuja essential oil

2.2. Identification of the volatile compounds

The identification of the volatile compounds in thuja essential oil should be done by the GC-MS method.

The analysis of the sample taken in the study was carried out using the Agilent Technology 7820A gas chromatograph (AGILENT Scientific, Santa Clara, CA, USA), coupled with the MSD 5975 mass spectrometer and equipped with a DB WAX capillary column (30 mx 250 pm x 0.25 p.m). The gas used was helium with a mass flow rate of 1 ml/min.

For the separation of the compounds, the following oven program was used: 40 °C for 1 min, 5 °C min-1 to 210 °C for 5 min. Injector and ion source temperatures were 250 and 150 °C, respectively. The injection volume was 1 µl of each pure, solvent-free oil or mixture with a 1:20 split ratio.

The NIST spectrum library was used to identify the volatile compounds. Identification was made by comparing the mass spectra with those stored in the NIST 02, Wiley 275 libraries. The percentage value of the individual components was calculated based on the GC peak areas without using correction factors.

2.3. The testing of the antimicrobial effect

The testing of the antimicrobial effect of thuja essential oil was carried out by the disc method, according to the Standard Norms for Testing the Antimicrobial Sensitivity of Impregnated Discs.

To test the antimicrobial effect of thuja essential oil, catalog strains from two Gram-negative bacterial species (*Proteus vulgaris* and *Salmonella typhimurium*) and two Gram-positive species (*Staphylococus aureus* and *Bacillus subtilis*) were used.

From the tested bacterial species, they initially prepared young 24-hour cultures as follows: on a Petri plate with nutrient agar, seeds were made with the bacteriological loop to obtain isolated colonies.

They were incubated at the thermostat for 24 hours at 37 °C, and from each culture corresponding to the bacterial species used in the test, a colony with a bacteriological loop was taken and passed into 10 ml of nutrient broth.

These tubes with broth were then incubated at a thermostat for 24 hours at 37 °C, being considered fresh and pure cultures.

From each bacterial species chosen for testing, dilutions were then made to obtain a cell density of 10^7/ml. Density was checked with the McFarland scale.

In order to test the culture medium, the nutrient agar was poured into sterile Petri dishes. One plate was used for each bacterial culture.After the solidification of the medium, the plates were kept at the thermostat for approximately 15 minutes to eliminate the condensation that forms on the cover of the plates due to the temperature difference between the plate and the culture medium. Then, 1 ml of each prepared culture was sown on the surface of the culture medium distributed in plates.

Through rotating movements, the uniform distribution of the culture on the surface of the environment was achieved. The excess liquid was aspirated. The plates were then left to rest for 15 minutes for the bacterial bodies to make contact with the culture medium.

Sterile Whatman discs (6 mm) were used and loaded with 20 µl of thuja essential oil and deposited on the inoculated culture medium. The plates were incubated at 37°C for 24 h.

The antimicrobial activity of thuja essential oil was studied at three concentrations: 25%, 50%, 75% essential oil.

The plates were incubated for 24 hours at 37°C, after which the diameter of the zone of inhibition was measured and expressed in mm.

The negative control was prepared using DMSO as the solvent, and amoxicillin as the positive control. All determinations were made in triplicate and for the statistical analysis the software was used to evaluate a one-way analysis of variance (ANOVA) at $p \leq 0.05$.

3. Results and discussion

3.1. Compounds identified

Following the gas chromatographic analysis coupled with mass spectrometry, the following compounds presented in table 1 and figure 2 were identified for thuja essential oil.

Table 1.
Volatile compounds identified in thuja essential oil

Compound	R.I	Conc.%
Monoterpene hydrocarbons		
1 α -Pinene	935	22.25
Camphene	947	1.32
Sabine	970	0.54
β -Pinene	974	0.88
β -Myrcene	992	2.16
3-Hulls	1012	20.47
p-Cymene	1020	0.49
Limonene	1024	3.38
Terpinolene	1092	4.58
Oxygenated monoterpenes		
terpinene-4-ol	1172	0.58
α-Terpinyl acetate	1280	2.72
Sesquiterpene hydrocarbons		
β -Element	1386	0.36
α-Cedrene	1412	0.81
β -Caryophyllene	1416	6.12
α-Humulene	1452	5.57
γ -Cadinene	1476	0.40
δ -Cadinene	1479	0.81
Oxygenated sesquiterpenes		
Spathulenol	1511	1.12
α-Cadinol	1541	0.67
Caryophyllene oxide	1581	0.62
Cedrol	1654	19,21

Following the gas chromatographic analysis, 21 components were identified in thuja essential oil, which add up to 95.06% of the total essential oil composition and represent four different groups of hydrocarbons, namely; monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons and oxygenated sesquiterpenes.

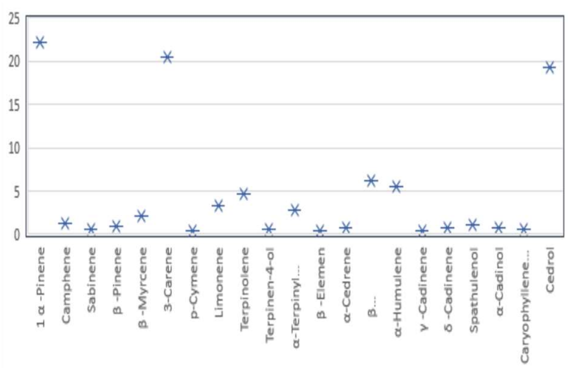

Figure 2. Compounds identified in Thuja essential oil

Monoterpene hydrocarbons constituted the most dominant chemical group (56.07%) and among the components α-pinene (22.25%) is the most predominant followed by 3-carenes (20.47), terpinolene (4.58), limonene (3.38), β-myrcene (2.16) and camphene (1.32).

Oxygenated monoterpenes account for 3.3% of thuja essential oil components, and the largest proportion was α-terpinyl acetate (2.72).

Sesquiterpene hydrocarbons accounted for 14.07%, including as predominant compounds β -caryophyllene (6.12) and α-humulene (5.57).

Oxygenated sesquiterpenes represent 21.62% of thuja essential oil composition and had cedrol (19,21) and spathulenol (1,12) as major components.

The composition of the essential oil showed some similarities with previous studies by other researchers, but with differences in the region where the plant was grown.

For example, the major components in the essential oil of *T. orientalis* from Iran are: pinene (21.9%), cedrol (20.3%), D-3-carene (10.5%), and limonene (7, 2%), while in the one from the Himalayas, the major constituents are: pinene (29.2%), D-3-carene (20.1%), cedrol (9.8%), caryophyllene (7.5%) and limonene (5.4%). These differences are due to genetic variability, climate conditions, harvesting season, soil composition and drying process [21].

Another study carried out in Pakistan showed that thuja essential oil has 22 main components represented by: α-pinene (40.6%), beta-caryophyllene (6.8%), cedrol (10.7%), alloaromadendrene (7.8%) and β -myrcene (3.7 %) and R-+-limonene (3.2 %) and another study carried out in Egypt highlights 23 main components, the most predominant being α pinene (21.83%), β- pinene (6.71%), β-caryophyllin (12.07), α-cedrol (6.86%), β-selinene (6.15%), limonin (5.49%) [11, 18].

3.2. The in vitro antimicrobial activity

The in vitro antimicrobial activity of thuja essential oil against some microorganisms was evaluated qualitatively and quantitatively by the microdilution method. The diameters of the zones of inhibition produced by thuja essential oil against the tested microorganisms are shown in table 2 and figure 3.

Table 2.
Diameters of inhibition zones produced by Thuja essential oil

Inhibition zone halo diameter (mm)				
Gram positive				
Conc.	**25%**	**50%**	**75%**	**Amox**
B. subtilis	12.6±0.14	14.8±0.11	30.12±0.16	39.4±0.11
St. aureus	21.8±0.12	24.18±0.12	35.42±1.08	N/A
Gram negative				
P. vulgaris	N/A	N/A	18.05±0.7	42.2±0.32
S. typhimurium	N/A	N/A	N/A	15.4±0.18

Amox – amoxicillin;

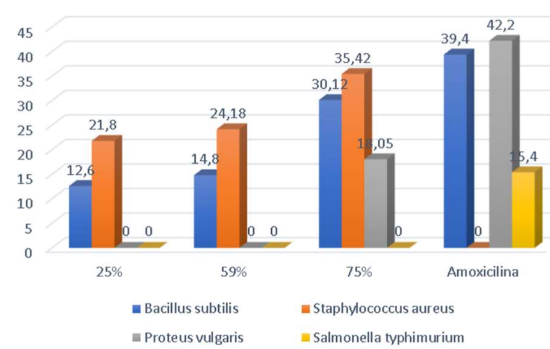

Figure 3. Graphical representation of the zone of inhibition (mm) at the three concentrations of thuja essential oil

The results obtained for thuja essential oil showed that it has the highest activity against gram positive *Staphylococcus aureus* (35.42±1.08) and *Bacillus subtitilis* (30.12±0.16), while it does not showed no activity against *Salmonella typhimurium* (gram negative), and in the case of the gram negative pathogen *Proteus vulgaris*, antimicrobial activity was obtained only at the maximum applied concentration (18.05±0.7 compared to 42.2±0.32 at amoxicillin).

The results obtained in this study are similar to those of other researchers who support the antimicrobial activity of thuja essential oil, especially against gram-positive pathogens [15, 20].

In another in vitro study, the strong antimicrobial activity of the methanolic extract of T. occidentalis against some Gram-negative and Gram-positive bacteria (*Escherichia coli, Citrobacter, Shigella flexenari, Yersinia aldovae, Staphylococcus aureus, Pseudomonas aeruginosa*) was highlighted. and fungi (*Saccharomyces cereviciae*, *Aspergillus parasiticus*, *Trichophyton rubrum*, *Yersinia aldovae* and *Candida albicans*) [16, 19].

Constituents of *T*. *occidentalis* essential oil such as: sabinyl acetate, fenchone, sabiene, β-thujone, α-pinene and terpinen-4-ol have been shown to have antimicrobial activity against *S*. *aureus*, *E. coli, E faecalis*, and α-thujone and β-thujone are very active against Gram-negative bacteria (*Pseudomonas aeruginosa* and *Klebsiella pneumonia*).

In studies to determine the inhibitory effect of *T. occidentalis* on *Pseudomonas aeruginosa* using the optical density technique, it was shown that aqueous and alcoholic extracts of *T. occidentalis* were effective against *P. aeruginosa* at a concentration of 50%, while at a concentration of 10% these extracts were less effective.

The antibacterial and antimicrobial activity of the essential oils obtained from the leaves and cones of *T. occidentalis* was tested against seven microorganisms: *Staphylococcus aureus*, *Aeromonas hydrophila*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella thyphimurium*, *Aspergillus flavus* and *Aspergillus niger*.

Both oils were effective, but higher microbial activity was observed with the essential oil obtained from the leaves. The antifungal effect was the strongest. This property of the oils is believed to be due to pinene, which is the major component of both oils [8, 14, 17].

4. Conclusions

Following the study carried out on the commercially procured thuja essential oil, regarding chromatographic analysis and antimicrobial efficacy testing, we can conclude that: Thuja essential oil has significant inhibitory effects against gram-positive and gram-negative bacteria, which are associated with clinical diseases; it is more effective against gram positives than gram negatives; ineffective against Salmonella typhimurium and the major components identified were: α–pinene, 3-carene, terpinolene, limonene, β-myrcene and camphene.

The presence of these compounds shows that thuja essential oil has a great potential for use in many medical applications.

Bibliography

1. Alves, L.D.S., Figueirêdo, C.B.M., Silva, C.C.A.R., Marques, G.S., Ferreira, P.A., Soares, M.F.R., Silva, R.M.F., Rolim-Neto, P.J., **2014**, *Thuja Occidentalis L. (Cupressaceae), Reviw of Botanical, Phytochemical, Pharmacological and Toxicological Aspects*, International Journal of Pharmaceutical Scienses and Research 5(4):1163-1177.
2. Aragón, Z., **2005**, *Lignanos (I): estructura y funciones en las plantas*, Revista de fitoterapia, 5 (1): 55-68.
3. Bellili, S., Aouadhi, C., Dhifi, W., Ghazghazi, H., Jlassi, C., Sadaka, C., El Beyrouthy, M., Maaroufi, A., Cherif, A., Mnif, W., **2018**, *The Influence of Organs on Biochemical Properties of Tunisian Thuja occidentalis Essential Oils,* Symmetry,*10*(11):649
4. Bushra, M., **2017**, *Study of the inhibitory effect of Thuja occidentalis against Peudomonas aeruginosa isolated from surgical wounds in vitro and in vivo*, International Journal of Science and Nature, 8(2):352-357

5. Dubey, S.K., Batra, A., **2008**, *Hepatoprotective activity from ethanol fraction of Thuja occidentalis Linn*., Asian Journal of Research in Chemistry, 1(1): 32-35.

6. Dubey, S.K., Batra, A., **2009**, *Role of phenolic compound rich ethanol fraction of Thuja occidentalis Linn. in protective mechanism*, Journal of Pharmacy Research 2(2): 217-225.

7. Dut Jasuja, N., Sharma, S., Choudhary, J., Joshi, S.C., **2013**, *Essential Oil and Important Activities of Thuja orientalis and Thuja occidentalis*, Journal of Essential Oil Bearing Plants, 18(4):931-949

8. Gupta, G., Srivastava, A.K., **2002***, In vitro activity of Thuja occidentalis Linn. against human pathogenic Aspergilla*, The Homephathic Heritage, 27(1): 5-12.

9. Hoffmann, D., **2003**, *Medical Herbalism: Principles and Practices*, Healing Arts Press p.588.

10. Hold, K.M., Sirisoma, N.S., Ikeda, T., Narashashi, T., Casida, J.E., **2000**, *Alpha-thujone (the active component of abshinte): gamma-aminobutyric acid type A receptor modulation and metabolic detoxification*, Proc. Natl. Acad. Sci. U.S.A., 97(8):3826-31.

11. Ibrahim, Magda T., Nevein, M., Abdel-Hady., Lamiaa, N., Hammad., **2004**, *GC/MS Analysis and biochamical studies of the essential oil of Thuja Orientalis L. growing in Egypt*. Bull. Fac. Pharm. Cairo Univ., Vol. 42, No. 1.

12. Jahan, N., Ahmad, M., Zia-Ul-Haq M., Alam, S., Qureshi, M., **2010**, *Antimicrobial screening of some medicinal plants of Pakistan,* Pakistan Journal of Botany 42(6): 4281-4284.

13. Johnston, WF., **2008**, *Thuja occidentalis, Conifers. Silvics of North America*, Washington, D.C.

14. Kéïta, S.M., Vincent, C., Schmidt, J.P., Arnason, J.T., **2001**, *Insecticidal effects of Thuja occidentalis (Cupressaceae) essential oil on Callosobruchus maculatus [Coleoptera:Bruchidae],* Canadian Journal of Plant Sciense, 81(1):173-177.

15. Lis, A., Liszkiewicz, R., Krajewska, A., **2016**, *Comparison of chemical composition of the essential oils from different parts of Thuja occidentalis L. 'Brabant' and T. occidentalis L. 'Smaragd*', Herba Polonica, 62(3):20-27.

16. Mohamad, J. K., Ghaytha, M., Bahira, Z., **2016**, *Antibacterial and Phytochemical Investigation of Thuja orientalis (L.) Leaves Essential Oil from Syria*, IJCPR, Volume 7(5).

17. Naser, B., Bodinet, C., Tegtmeier, M., Lindequist, U., **2005**, *Thuja occidentalis (Arbor vitae): A Review of its pharmaceutical, pharmacological and clinical properties*, Evidence-based complementary and alternative medicine, (1): 69-78.

18. Riaz, M., Rashid, M. K., Chaudary, F. M., **2005**, *Volatile Constituents of the Leaves Pakistani Cumpressus sempervirens and Thuja orientalis,* Pakistan Journal of Science and Industrial Research, Vol. 42, No. 2, pp.98-101

19. Shiv Nandan, S., Sunil, R., Man Kumar, T., **2017**, *Antibacterial Effects of Thuja Leaves Extract*, International Journal of Applied Sciences and Biotechnology, 5(2):256-260.

20. Tsiri, D., Graiku, K, Poblock-Olech, L., Krauze-Baranows, M., Spyropoulos, C., Chinou, I., **2009**, *Chemosystematic Value of the Essential Oil Composition of Thuja species Cultivated in Poland – Antimicorbial Activity*, Molecules 14(11):4707-4715.

21. Wajaht, A.S., Mahpara, Q., **2014**, *Chemical composition, Antioxdant and Antibacterial activity of Thuja Orientalis essential oil.* World Journal of Pharmaceutical Sciences, 2(1): 56-61.