CHEMISTRY JOURNAL OF MOLDOVA. General, Industrial and Ecological Chemistry. 2021, 16(2), 83-90
ISSN (p) 1857-1727 ISSN (e) 2345-1688
http://cjm.asm.md
http://dx.doi.org/10.19261/cjm.2021.877

CHEMICAL COMPOSITION OF THE ESSENTIAL OIL AND ANTIMICROBIAL PROPERTIES OF CRUDE EXTRACT FROM *TANACETUM CORYMBOSUM* (L.) SHI. BIP.

Alexandru Ciocarlan [a*], Lucian Lupascu [a], Aculina Aricu [a], Ion Dragalin [a], Nina Ciocarlan [b], Inga Zinicovscaia [a,c,d], Valerina Slanina [e], Nikita Yushin [c]

[a]*Institute of Chemistry, 3, Academiei str., Chisinau MD 2028, Republic of Moldova*
[b]*National Botanical Garden (Institute) "A. Ciubotaru", 18, Padurii str., Chisinau MD 2002, Republic of Moldova*
[c]*Joint Institute for Nuclear Research, 6, Joliot-Curie str., 1419890, Dubna, Russia*
[d]*Horia Hulubei National Institute for R&D in Physics and Nuclear Engineering, 30, Reactorului str., Bucharest, Magurele, Romania*
[e]*Institute of Microbiology and Biotechnology, 1, Academiei str., Chisinau MD 2028, Republic of Moldova*
[*]*e-mail address:* algciocarlan@yahoo.com; alexandru.ciocarlan@ichem.md

In memoriam of Professor Mihai Coltsa at 75th anniversary

Abstract. The aim of the present study was to determine the chemical composition of the essential oil and the evaluation of the antimicrobial activity of ethanolic extract from *T. corymbosum* plants from the Republic of Moldova. Hydrodistillation and Soxhlet extraction have been used to obtain volatile oil samples and extracts from plants, respectively. The components of volatile oil were identified by GC-MS analyses. The antibacterial activity of the extracts was assessed by the successive double dilution method. The GC-MS analysis revealed the presence of 38 compounds, including terpenes - germacrene D (33.3% and 47.5%), (*Z*)-*β*-farnesene (8.6% and 16.1%), *γ*-elemene (3.1% and 5.2%), *β*-caryophyllene (4.2% and 6.5%), aliphatic - palmitic (2.2% and 7.1%) and linoleic (0.2% and 1.0%) fatty acids, fatty alcohol *n*-octadecanol (0.6% and 9.7%), higher alkane *n*-heneicosane (1.0% and 6.9%) as the major constituents. The *in vitro* assessments of hydroacoholic extract against five bacterial strains and two fungal species showed its promising antibacterial/antifungal activities at 0.03% and 0.015%, respectively. According to the obtained data, the *T. corymbosum* species that grows in Moldova belongs to the germacrene D chemotype. This species holds great potential to be used as an herbal antibacterial agent.

Keywords: chemical composition, ethanolic extract, essential oil, Corymbflower tansy, antimicrobial activity.

Received: 11 September 2021/ Revised final: 28 November 2021/ Accepted: 03 December 2021

Introduction

The genus *Tanacetum* L. belongs to Asteraceae family, one of the largest families of dicotyledonous plants [1]. According to different authors, the genus *Tanacetum* comprises about 150-200 species distributed in the Northern Hemisphere (Europe, temperate Asia, North Africa, and America) [2,3]. Unlike other *Tanacetum* species, Corymbflower tansy (*Tanacetum corymbosum* (L.) Sch. Bip. (syn.: *Pyrethrum corymbosum* (L.) Scop., *Chrysanthemum corymbosum* L.,) was less studied. In the flora of the Republic of Moldova, *Tanacetum* L. species are represented by 4 species, including *Tanacetum corymbosum* (L.) Sch. Bip. [4]. Extracts from plants with various therapeutic purposes, are known and used since antiquity. The species of the genus *Tanacetum* are also part of this series and have been used in folk medicine since ancient times for the treatment of respiratory, digestive, skin, allergic, excretory and reproductive systems, central nervous and locomotor systems disorders [5-9]. Due to the mentioned above properties, the *Tanacetum* species are also used as cosmetics, insecticides, balsams, dyes, food preservatives, flavouring agents, and herbal remedies [10,11].

In the last decades, a large number of chemical components of *Tanacetum* species have been extracted and identified, such as terpenes and terpenoids, which are the main constituents of the essential oils and extracts, as well as polyphenols, especially flavonoids, phytosterols, phenolic acids, sesquiterpene lactones, bitter substances, pyrethrins, coumarins and tannic acid [9,11,12-18]. The complex chemical composition

of *Tanacetum* L. species produces a wide range of biological activities, such as antifeedant [19], antioxidant [20-22], immunomodulatory [23], cytotoxic [21,22], anti-inflammatory [21,24], insecticide, antimicrobial [6,22,25] and antibacterial [26], *etc*. Moreover, in the case of *Tanacetum corymbosum* (*T. corymbosum)*, the whole herba or parts of it were reported as remedies for digestive disorders, particularly gastritis, as antiprotozoal, antibacterial and antioxidant remedies [27]. Also, the antimicrobial activity of its essential oils, anticoagulant and anti-fibrinolytic activities of the aqueous and chloroform extracts were reported [11]. Thus, scientific literature offers very limited information on the chemical composition of *Tanacetum corymbosum* essential oil. Compared to the chemical composition of other species of the genus *Tanacetum*, that of the species *T. corymbosum* reported in this paper is quite different.

The goal of the study was to evaluate the chemical composition of the essential oil from fresh and dried *T. corymbosum*, using GC-MS analysis and the *in vitro* antibacterial and antifungal activity of its ethanolic extract for further exploration of potential pharmaceutical applications of this species.

Experimental

Materials

All the solvents and reagents were of analytical grade. Anhydrous sodium sulphate, 96% ethanol and diethyl ether were obtained from Merck (Darmstadt, Germany). Deionized water produced by a Milli-Q Millipore system (Bedford, U.S.A.) was used to prepare aqueous solutions.

The plant material (*T. corymbosum* - aerial parts with inflorescences) was harvested in June 2018 in the Collection of Medicinal Plants of the National Botanical Garden (Institute), (Republic of Moldova) geographically located at N 46°58′ 25.43″, E 28°52′ 47.16″. The aerial parts (872 g) of Corymbflower tansy plants were dried at room temperature (20-23°C) in shadow. The identity of the species was established by Dr. Nina Ciocarlan (Department of Vegetal Resources) and Dr. Tatiana Izverscaia (Department of Spontaneous Flora and Herbarium). The voucher specimen (voucher No. 237243/2018) has been deposited in the Herbarium of the National Botanical Garden (Institute), Chisinau, the Republic of Moldova.

Methods

Extraction procedure of essential oil

The essential oil has been extracted from the fresh (sample A, 421 g) and air-dried (sample B, 111 g) plant material using an all-glass Neo-Clevenger-type apparatus. The samples (fresh and dried) were hydro-distilled separately for 3 h and then the distillates were supplementary extracted with diethyl ether. After phase separation, the etheric extracts were dried over anhydrous sodium sulphate and then used for chromatographic measurements.

Soxhlet extraction

The crushed dried plants (10 g) were extracted in a Soxhlet-type extractor with ethanol for 4 h. The hydroalcoholic extract was further filtered and distilled under reduced pressure up to dry. The crude extract (1.61 g, 16.1%) was used for the preparation of solutions for the antimicrobial studies.

GC-MS analysis

The GC-MS analysis was performed using an Agilent Technologies 7890A gas chromatograph coupled with a 5975C Mass Selective Detector (MSD) equipped with a split-splitless injector (1 μL). The analysis was carried out on a fused silica capillary HP-5MS calibrated column (30 m × 0.25 mm i.d.; film thickness 0.25 μm). The injector and detector temperatures were kept at 250°C. Helium was used as carrier gas at a flow rate of 1.1 mL/min; oven temperature program was 70°C/2min, which was then programmed to 200°C at the rate of 5°C/min, and finally to 300°C at the rate of 20°C/min; the split ratio was 1:50. The MSD ionization energy of 70 eV, scan time 1 s, acquisition mass range was from 30 to 450 amu, solvent delay 3 min.

Antimicrobial activity evaluation

The antimicrobial activity assessment of the ethanolic extract from the dried *T. corymbosum* plants was performed on the following microorganisms: non-pathogenic Gram-positive and Gram-negative strains of *Bacillus subtilis* NCNM-BB-01 (ATCC 33608) and *Pseudomonas fluorescens* NCNM-PFB-01 (ATCC 25323), respectively, phytopathogenic strains of *Xanthomonas campestris* NCNM BX-01 (ATCC 53196), *Erwinia amylovora* NCNM BE-01 (ATCC 29780), *E. carotovora* NCNM BE-03 (ATCC 15713) and fungus strains of *Candida utilis* NCNM Y-22 (ATCC 44638) and *Saccharomyces cerevisiae* NCNM Y-20 (ATCC 4117).

The successive double dilution method was used for testing. According to this method after the first day of testing is determined the minimal inhibitory concentration (MIC) of the preparation. For the estimation of the minimum bactericidal concentration (MBC) and minimum fungicidal

concentration (MFC), the contents of the test tubes with MIC and with higher concentrations were seeded on peptone and Sabouraud agar from Petri dishes. The concentration of preparation, which does not allow the growth of any colony of microorganism, was considered to be its minimal bactericidal and fungicidal concentrations [28].

Results and discussion

Chemical composition of the essential oil

According to the literature data, the chemical composition of the volatile part isolated from *Tanacetum* species is highly variable not only at the species level but also at the subspecies level. This chemical variation is also influenced by its geographical origin. For this reason, the species chemotype is identified according to the main constituent of the essential oil. In this regard, authors reported germacrene and spathulenol as major components of essential oil of *T. zahlbruckneri* [29], 1,8-cineol, caryophyllene oxide, hexadecanoic acid, decanoic acid, spathulenol and linalool oxide acetate for *T. tabrisianum* [29], *α*-thujone, camphor, borneol, 1,8-cineole and *β*-thujone for *T. polycephalum* [30], a series of unsaturated aldehydes and fatty acids for *T. chiliophyllum* [7], bornyl acetate, pinocarvone, camphor and terpinolene for *T. balsamita* [31], camphor, 1,8-cineol, *α*- and *β*-thujone, borneol, camphene, *p*-cimene for *T. vulgare* [32,33], *α*- and *β*-thujone for *T. messicytus* [34]; borneol and 1,8-cineol for *T. praeteritum* [34] and caryophyllene oxide and *α*-thujone for *T. argyrophyllum* [35].

Only several compounds from the list shown in Table 1 are reported as major constituents of essential oil obtained from *T. vulgare*, *T. polycephalum* [11], *T. argenteum* [36], *T. zahlbruckneri* and *T. tabrisianum* [29], such as: camphene, *β*-caryophillene, caryophillene oxide, *δ*- and *γ*-cadinene, germacrene D, spathulenol, *α*-cadinol, heptacosane, *n*-hexadecanoic acid and *n*-octadecanol. It was identified only one research article regarding the chemical composition of the volatile oil from *T. corymbosum* [37]. In this source it is mentioned that the main constituents of *T. corymbosum* essential oil are sesquiterpene hydrocarbons *α*-cadinene (50.9%) and *β*-cadinene (15.1%), from a total of 41 detected compounds, of which 33 were identified as mono- and sesquiterpenoids [37].

The chemical composition of *T. corymbosum* L. oil (samples A and B) and the retention time of the identified component and their contents are shown in Table 1. A total of 38 (sample A) and 22 (sample B) constituents, representing 99.5% and 95.6%, respectively, were detected. The essential oil of *T. corymbosum* of Moldavian origin contains two isomeric *δ*-cadinene (0.6%, sample A) and *β*-cadinene in both samples (2.0% and 1.5%), but generally, its chemical composition is quite different from the one reported above for *Tanacetum* spp. and includes many unreported compounds.

The GC-MS analysis of *T. corymbosum* oil resulted in identification of 38 components (see Figure 1 and Table 1).

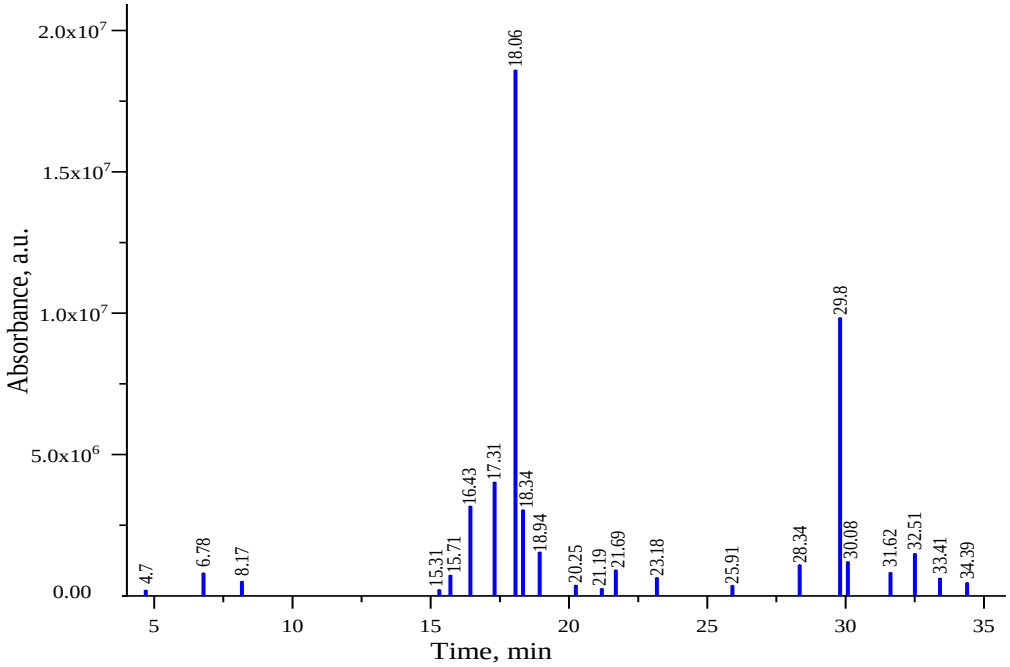

Figure 1. Chromatographic profile of *T. corymbosum* essential oil.

Table 1

Chemical composition (%) of *T. corymbosum* essential oil.

No.	*RT*[*] *(min)*	*Component*	*Sample*[**]*, content (%)*	
			A	*B*
1	4.70	Camphene	0.2	-
2	6.78	(*Z*)-*β*-Ocimene	0.9	-
3	8.17	Nonanal	0.7	-
4	15.31	Copaene	0.3	-
5	15.71	*β*-Elemene	1.0	0.9
6	16.43	*β*-Caryophyllene	4.2	6.5
7	16.66	*epi*-Bicyclosesquiphellandrene	0.3	-
8	17.31	(*Z*)-*β*-Farnesene	8.6	16.1
9	17.61	(4*Z*)-4-(2,2-Dimethyl-6-methylenecyclohexylidene)-3-methylbutan-2-one	0.8	-
10	18.06	Germacrene D	47.5	33.3
11	18.34	*γ*-Elemene	5.2	3.1
12	18.53	*α*-Farnesene	1.6	1.2
13	18.66	9-Cedranone	0.4	0.6
14	18.73	(+)-*δ*-Cadinene	0.6	-
15	18.94	*β*-Cadinene	2.0	1.5
16	20.25	Spathulenol	0.5	0.7
17	20.38	Cariophyllene oxide	0.5	3.3
18	20.58	*γ*-Gurjunene	0.5	-
19	21.19	Humulane-1,6-dien-3-ol	0.4	-
20	21.38	Cubenol	0.3	-
21	21.69	(*E*)-Muurolol	1.6	0.9
22	21.78	*δ*-Cadinol	0.4	-
23	21.98	*α*-Cadinol	1.7	1.3
24	23.18	*n*-Tetradecanal	0.8	0.8
25	25.91	Hexahydrofarnesyl acetone (phytone)	0.5	1.6
26	28.34	*n*-Hexadecanoic acid (palmitic)	2.2	7.1
27	29.07	*n*-Octadecanal	0.2	-
28	29.80	*n*-Octadecanol	9.7	0.6
29	30.08	(*E*)-Phytol	1.4	2.0
30	30.28	1,7-Octadecynoic acid	0.2	-
31	30.34	12-Methyl-(*E*,*E*)-2,13-octadecadien-1-ol	0.4	-
32	30.44	(*Z*,*Z*)-9,12-octadecadienoic acid (linoleic)	0.2	1.0
33	30.91	(*Z*)-Lanceol	0.1	0.7
34	31.38	*n*-Docosanol (behenic alcohol)	1.2	-
35	31.62	Eicosanoic acid methyl ester (methyl arachidate)	0.6	-
36	32.51	*n*-Heneicosane	1.0	6.9
37	33.41	*n*-Heptacosane	0.4	2.9
38	34.39	*n*-Octacosane	0.4	2.6
Class		*Subclass*	*Sample, content (%)*	
			A	*B*
Terpenic compounds			*80.2*	*72.1*
		Monoterpenes	*1.1*	-
		Monoterpene hydrocarbons	1.1	-
		Oxygenated monoterpenes	-	-
		Sesquiterpenes	*77.7*	*70.1*
		Sesquiterpene hydrocarbons	71.8	62.6
		Oxygenated sesquiterpenes	5.9	7.5
		Diterpenes	*1.4*	*2.0*
		Oxygenated diterpenes	1.4	2.0
Aliphatic compounds			*19.3*	*23.5*
		Hydrocarbons	1.8	12.4
		Alcohols	11.3	0.6
		Aldehydes, ketones	3.0	2.4
		Acids	2.6	8.1
		Esters	0.6	-
		Total	**99.5**	**95.6**

[*]*RT - retention time;*

[**]*Samples of essential oil extracted from fresh (A) and dry (B) plant material.*

According to the data shown in Table 1, the chemical composition of the essential oil from fresh and dry plant material of *T. corymbosum* growing in Moldova is quite varied and consists mainly of compounds belonging to different classes of terpenic and aliphatic compounds.

The sesquiterpene hydrocarbons are the main compounds in the composition of the essential oil from *T. corymbosum* growing in Moldova. Their content does not differ too much and constitutes 71.8% for sample A and 62.6% for sample B, which confirms that during drying, some of the sesquiterpene hydrocarbons are lost, *e.g.* copaene, *epi*-bicyclosesquiphellandrene, δ-cadinene and γ-gurjunene. The results obtained show that the species *T. corymbosum* growing in Republic of Moldova belongs to germacrene D chemotype, this being its dominant constituent, 47.5% and 33.3%, respectively, followed by (*Z*)-β-farnesene (8.6% and 16.1%), γ-elemene (5.2% and 3.1%) β-caryophyllene (4.2% and 6.5%), and β-cadinene (2.0% and 1.5%).

Oxygenated sesquiterpenes make up the following group of terpene constituents with a content of 5.9% (sample A) and 7.5% (sample B), respectively. These are represented mostly by alcohols, such as: α-cadinol (1.7% and 1.3%), (*E*)-muurolol (1.6% and 0.9%) and spathulenol (0.5% and 0.7%) identified in both samples, while humulane-1,6-dien-3-ol (0.4%), cubenol (0.3%) and δ-cadinol (0.4%) were identified only in sample A. This could be explained by the chemical transformation (*e.g.* oxidation, isomerization or others) of some minor compounds during drying. There were also found oxygenated derivatives such as caryophyllene oxide (0.5% and 3.3%), and ketone 9-cedrone (0.4% and 0.6%). It should be noted that the monoterpene fraction was identified only in fresh oil and is represented by camphene (0.2%) and (*Z*)-β-ocimene (0.9%). This confirms the above hypothesis on the changes that the raw material undergoes during the drying process. It is noteworthy that oxygenated diterpene (*E*)-phytol (1.4% and 2.0%) were present in a higher amount than monoterpenes. Aliphatic compounds represent the second group of compounds in the composition of oil (19.3% and 23.5%, respectively). Higher alkanes are represented by heinecosane (1.0% and 6.9%), heptacosane (0.4% and 2.9%) and octacosane (0.4% and 2.6%) and their content is quite high especially in sample B (12.4%). Higher alcohols are highlighted more strongly in sample A (11.3%), and are represented by octadecan-1-ol (9.7% and 0.6%), 12-methyl-(*E*,*E*)-2,13-octadecadien-1-ol (0.4%, sample A) and docosanol-1 (1.2%, sample A).

Carbonyl compounds have a low content, as follow, in sample A: nonanal (0.7%), (4*Z*)-4-(2,2-dimethyl-6-methylenecyclohexylidene)-3-methylbutan-2-one (0.8%), octadecanal (0.2%) and tetradecanal (in both 0.8%). These visible variations of alcohols and carbonyl compounds contents in samples A and B are probably due to the oxidation processes which occur during drying and storage of vegetal material.

The saturated and unsaturated fatty acids and their esters are represented by *n*-hexadecanoic acid (2.2% and 7.1%), linoleic acid (0.2% and 1.0%), 1,7-octadecynoic acid (0.2% in A) and eicosanoic acid methyl ester (0.6% in A). The previous remark regarding the content is also valid for carboxylic constituents of samples A and B, their content increases due to the oxidation of lower classes of constituents; as to the ester, hydrolysis has possibly occurred.

Antimicrobial activity of the extract

The moderate antimicrobial activity against Gramm-positive bacteria (MIC 3.12 mg/mL), moderate antioxidant activity on both, DPPH radical-scavenging and reducing power methods at EC_{50} of 344±2.6 μg/mL and 116.80±0.94 μg/mL and high cytotoxic activity against the human HeLa cervical cancer cell line (93.71%, at 200 μg/mL) and normal African green monkey kidney (Vero) epithelial cell line (96.98%, at 200 μg/mL) of the methanolic extract obtained from *T. corymbosum* plants have been reported [22]. The neutron activation analysis (NAA) of *T. corymbosum* plant material showed the presence of four macroelements and 17 microelements [38].

Phytopathogenic bacteria can cause various diseases (bacteriosis) affecting agricultural plants, for example: bacterial fire blight disease, soft rot of economically important crops such as potatoes, tomatoes and cucumbers, bacterial bleaching of tomatoes (*Solanum lycopersicum* L.) and pepper (*Capsicum annuum).* For this reason, the identification of natural compounds with antibacterial properties remains a problem of current importance.

The *in vitro* assessment of ethanolic extract from dried *T. corymbosum* plants (Table 2) of Moldavian origin have shown high antibacterial activity against both non-pathogenic Gram-positive/Gram-negative (*Bacillus subtilis* and *Pseudomonas fluorescens*) and phytopathogenic (*Xanthomonas campestris*, *Erwinia amylovora*, *E. carotovora*) bacteria in the range of concentrations of 0.03-0.06%.

Table 2

The antimicrobial activity of the ethanolic extract from dried *T. corymbosum* plants.

Tested microorganisms	*Double successive dilutions (MBC, MFC, %)*							
	0.25	*0.12*	*0.06*	*0.03*	*0.015*	*0.007*	*0.0035*	*0.0017*
Bacillus subtilis CNMN BB-01 (4.8 x 10^8 CFU/mL)	-	-	-	-	+	+	+	+
Pseudomonas fluorescens CNMN-PFB-01(4.8 x 10^8 CFU/mL)	-	-	-	+	+	+	+	+
Xanthomonas campestris (4.8 x 10^8 CFU/mL)	-	-	-	-	+	+	+	+
Erwinia amylovora (4.8 x 10^8 CFU/mL)	-	-	-	+	+	+	+	+
Erwinia carotovora (4.8 x 10^8 CFU/mL)	-	-	-	-	+	+	+	+
Candida utilis (3.0 x 10^7 CFU/mL)	-	-	-	-	-	+	+	+
Saccharomyces cerevisiae (3.0 x 10^7 CFU/mL)	-	-	-	-	+	+	+	+

MBC - minimum bactericidal concentration;
MFC - minimum fungicidal concentration.

Its antifungal properties against *Candida utilis* and *Saccharomyces cerevisiae* strains lie in the range of concentrations of 0.015-0.03% (Table 2).

The pronounced antimicrobial activity of the extract can be explained by the significant content of flavonoids and especially of sesquiterpene lactones. It is well known that this class of terpenoids is characterized by a high antimicrobial activity [42,43]. The main mechanism of action of the extract includes the disruption of the cell membrane that causes leakage of considerable amounts of cellular constituents, *e.g.* proteins and sugars. It can also cause a decrease in cellular weight with significant changes in the permeability of the membrane as confirmed by microscopy tests [44].

Conclusions

This is the first study devoted to the determination of the chemical composition of the essential oil obtained from fresh and dried *T. corymbosum* plants growing in the Republic of Moldova. The gas-chromatographic analysis allowed the identification of 38 constituents of essential oil, most of these being reported for the first time and obtained data revealed that the species belongs to germacrene D chemotype, which is the major constituent (33.3-47.5%).

The *in vitro* assessment of the hydro-ethanolic extract obtained from dried *T. corymbosum* plants on seven strains of microorgamisms confirmed its high antibacterial and antifungal activity in a range of 0.015-0.03%.

The data obtained may be useful both for researchers and for producers interested in new or less studied species of medicinal plants in healthcare and their biological activities.

Acknowledgements

L.L. is grateful to the Institute of Genetics, Physiology and Plant Protection and the National Collection of Non-Pathogenic Microorganisms of the Institute of Microbiology and Biotechnology for the offering of the microbial strains for testing.

Funding

This research was funded by the National Agency for Research and Development, project PLANTERAS 20.80009.8007.03 “New substances with preventive and therapeutic potential based on natural compounds of plant origin and modern methods of organic synthesis”.

References

1. Tutin, T.G.; Heywood, V.H.; Burges, N.A.; Moore, D.M.; Valentine, D.H.; Walters, S.M.; Webb, D.A. Eds. Flora Europaea. Cambridge University Press: New York, 1976, pp. 169-171.
2. Stevens, P.F. Angiosperm Phylogeny website. Version 14, 2017. www.mobot.org/MOBOT/research/APweb
3. Bremer, K.; Humphries, C.J. Generic monograph of the *Asteraceae-Anthemideae*. Bulletin oh the Natural History Museum. Botany series, 1993, 23(2), pp. 71-177. https://www.biodiversitylibrary.org/page/2322617#page/19/mode/1up
4. Negru, A. The determinator of plants from the flora of the Republic of Moldova. Universul Publishing House: Chisinau, 2007, 391 p. (in Romanian).
5. Lahlou, S.; Tahraoui, A.; Israili, Z.; Lyoussi, B. Diuretic activity of the aqueous extracts of *Carum carvi* and *Tanacetum vulgare* in normal rats.

Journal of Ethnopharmacology, 2007, 110(3), pp. 458-463. DOI: https://doi.org/10.1016/j.jep.2006.10.005
6. Coelho de Souza, G.; Haas, A.P.S.; von Poser, G.L.; Schapoval, E.E.S.; Elisabetsky, E. Ethnopharmacological studies of antimicrobial remedies in the south of Brazil. Journal of Ethnopharmacology, 2004, 90(1), pp. 135-143. DOI: https://doi.org/10.1016/j.jep.2003.09.039
7. Kubo, A.; Kubo, I. Antimicrobial agents from *Tanacetum balsamita*. Journal of Natural Products, 1995, 58(10), pp. 1565-1569. DOI: https://doi.org/10.1021/np50124a013
8. Ambrožič-Dolinšec, J.; Kovač, M.; Zel, J.; Camloh, M. Pyrethrum (*Tanacetum cinerariifolium*) from the northern Adriatic as a potential source of natural insecticide. Annales. Series Historia Naturalis, 2007, 17(1), pp. 39-46.
9. Özbilgin, S.; Akkol, E.K.; Ergene Öz, B.; Ilhan, M.; Saltan, G.; Bahadir Acıkara, Ö.; Tekin, M.; Keleş, H.; Süntar, I. *In vivo* activity assessment of some *Tanacetum* species used as traditional wound healer along with identification of the phytochemical profile by a new validated HPLC method. Iranian Journal of Basic Medicinal Sciences, 2018, 21(2), pp. 145-152. DOI: https://doi.org/10.22038/IJBMS.2018.24258.6055
10. Baczek, K.B.; Kosakowska, O.; Przybyl, J.L.; Pioro-Jabrucka, E.; Costa, R.; Mondello, L.; Gniewosz, M.; Synowiec, A.; Weglarz, Z. Antibacterial and antioxidant activity of essential oils and extracts from costmary (*Tanacetum balsamita* L.) and tansy (*Tanacetum vulgare* L.). Industrial Crops and Products, 2017, 102, pp. 154-163. DOI: https://doi.org/10.1016/j.indcrop.2017.03.009
11. Kumar, V.; Tyagi, D. Chemical composition and biological activities of essential oils of genus *Tanacetum* – a review. Journal of Pharmacognosy and Phytochemistry, 2013, 2(3), pp. 159-163. https://www.phytojournal.com/archives/2013/vol2issue3/PartC/36.1.pdf
12. Başer, K.H.C.; Demirci, B.; Tabanca, N.; Özek, T.; Gören, N. Compozition of the essential oils of *Tanacetum armenium* (DC.) Schultz Bip., *T. balsamita* L., *T. chiliophyllum* (Fisch & Mey) Schultz Bip. var. *chiliophyllum* and *T. haradjani* (Rech. Fil.) Grierson and the enantiomeric distribution of camphor and carvone. Flavour and Fragrance Journal, 2001, 16(3), pp. 195-200. DOI: https://doi.org/10.1002/ffj.977
13. Sanz, J.F.; Marco, J.A. NMR studies of Tatridin A and some related sesquiterpene lactones from *Tanacetum vulgare*. Journal of Natural Products, 1991, 54(2), pp. 591-596. DOI: https://doi.org/10.1021/np50074a040
14. Kumar, V.; Sharma, Y. Chemical composition and antibacterial activity of essential oils of *Tanacetum longifolium*. International Journal of Current Microbiology and Applied Sciences, 2016, 5(10), pp. 836-841. DOI: https://dx.doi.org/10.20546/ijcmas.2016.510.091
15. Todorova, M.N.; Evstatieva, L.N. Comparative study of *Tanacetum* species growing in Bulgaria. Zeitschrift fur Naturforschung C, 2001, 56(7-8), pp. 506-512. DOI: https://doi.org/10.1515/znc-2001-7-806
16. Long, C.; Sauleau, P.; David, B.; Lavaud, C.; Cassabois, V.; Ausseil, F.; Massiot, G. Bioactive flavonoids of *Tanacetum parthenium* revisited. Phytochemistry, 2003, 64(2), pp. 567-569. DOI: https://doi.org/10.1016/s0031-9422(03)00208-5
17. Palsson, K.; Jaenson, T.G.T.; Baeckström, P.; Borg-Karlson, A.K. Tick repellent substances in the essential oil of *Tanacetum vulgare*. Journal of Medical Entomology, 2008, 45(1), pp. 88-93. DOI: https://doi.org/10.1093/jmedent/45.1.88
18. Abad, M.J.; Bermejo, P.; Villar, A. An approach to the genus *Tanacetum* L. (Compositae): Phytochemical and pharmacological review. Phytotherapy Research, 1995, 9(2), pp. 79-92. DOI: https://doi.org/10.1002/ptr.2650090202
19. Susurluk, H.; Çalişkan, Z.; Gürkan, O.; Kirmizigül, S.; Gören, N. Antifeedant activity of some *Tanacetum* species and bioassay guided isolation of the secondary metabolites of *Tanacetum cadmeum* ssp. *cadmeum* (Compositae). Industrial Crops and Products, 2007, 26(2), pp. 220-228. DOI: https://doi.org/10.1016/j.indcrop.2007.04.002
20. Mantle, D.; Eddeb, F.; Pickering, A.T. Comparison of relative antioxidant activities of British medicinal plant species *in vitro*. Journal of Ethnopharmacology, 2000, 72(1-2), pp. 47-51. DOI: https://doi.org/10.1016/s0378-8741(00)00199-9
21. Coté, H.; Boucher, M.A.; Pichette, A.; Legault, J. Anti-inflammatory, antioxidant, antibiotic and cytotoxic activities of *Tanacetum vulgare* L. essential oil and its constituents. Medicines, 2017, 4(2), pp. 34-42. DOI: https://doi.org/10.3390/medicines4020034
22. Ivănescu, B.; Tuchiluş, C.; Cociorvă, A.; Lungu, C.; Mihai, C.T.; Gheldiu, A.-M.; Vlase, L. Antioxidant, antimicrobial and cytotoxic activity of *Tanacetum vulgare*, *Tanacetum corymbosum* and *Tanacetum macrophyllum* extracts. Farmacia, 2018, 66(2), pp. 282-288. https://farmaciajournal.com/wp-content/uploads/2018-02-art-13-Ivanescu_Tuchilus_Vlase_282-288.pdf
23. Xie, G.; Schepetkin, I.A.; Quinn, M.T. Immunomodulatory activity of acidic polysaccharides isolated from *Tanacetum vulgare* L. International Immunopharmacology, 2007, 7(13), pp. 1639-1650. DOI: https://doi.org/10.1016/j.intimp.2007.08.013
24. Schinella, G.R.; Giner, R.M.; Recio, M.C.; Mordujovich de Buschiazzo, P.; Rios, J.L.; Manez, S. Anti-inflammatory effects of South American *Tanacetum vulgare*. Journal of Pharmacy and Pharmacology, 1998, 50(9), pp. 1069-1074. DOI: https://doi.org/10.1111/j.2042-7158.1998.tb06924.x

25. Bagci, E.; Kursat, M.; Kosak, A.; Gur, S. Composition and antimicrobial activity of the essential oils of *Tanacetum balsamita* L. subsp. *balsamita* and *T. chiliophyllum* (Fisch. et Mey.) Schultz Bip. var. *chiliophyllum* (Asteraceae) from Turkey. Journal of Essential Oil Bearing Plants, 2008, 11(5), pp. 476-484. DOI: https://doi.org/10.1080/0972060X.2008.10643656
26. Masoudi, S.; Abbassi, J. Antibacterial activity and comparison of the volatile oils of *Tanacetum tenuisectum* (Boiss.) Podl. obtained by three different methods of extraction. Iranian Journal of Pharmaceutical Research, 2017, 16, pp. 188-196. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5963660/
27. Budantsev, A.L.; Lesiovskaia, E.E. Wild useful plants of Russia. SPCPA: Saint Petersburg, 2001, 663 p. (in Russian).
28. Method of serial dilutions in broth. Saint-Petersburg Pasteur Institute. (in Russian). http://www.dntpasteur.ru/metodic2_4_2_2.php/
29. Polatoğlu, K.; Demirci, B.; Gören, N.; Hüsnü Can Başer, K. Essential oil composition of endemic *Tanacetum zahlbruckneri* (Náb.) and *Tanacetum tabrisianum* (Boiss.) Sosn. and Takht. from Turkey. Natural Product Research, 2011, 25(6), pp. 576-584. DOI: https://doi.org/10.1080/14786419.2010.483434
30. Morteza-Semnani, K. Composition of the essential oil of *Tanacetum polycephalum* Schultz Bip. Journal of Essential Oil Research, 2006, 18(2), pp. 129-130. DOI: https://doi.org/10.1080/10412905.2006.9699041
31. Jaimand, K.; Rezaee, M.B. Chemical constituents of essential oils from *Tanacetum balsamita* L. ssp. *balsamitoides* (Schultz-Bip.) Grierson. from Iran. Journal of Essential Oil Research, 2005, 17(5), pp. 565-566. DOI: https://doi.org/10.1080/10412905.2005.9698996
32. Collin, G.J.; Deslauriers, H.; Pageau, N.; Gagnon, M. Essential oil of Tansy (*Tanacetum vulgare* L.) of Canadian origin. Journal of Essential Oil Research, 1993, 5(6), pp. 629-638. DOI: https://doi.org/10.1080/10412905.1993.9698296
33. Holopainen, M.; Hiltunen, R.; von Schantz, M. A study on Tansy chemotypes. Planta Medica, 1987, 53(3), pp. 284-287. DOI: https://doi.org/10.1055/s-2006-962707
34. Gören, N.; Demirci, B.; Başer, K.H.C. Composition of the essential oils of *Tanacetum* spp. from Turkey. Flavour and Fragrance Journal, 2001, 16(3), pp. 191-194. DOI: https://doi.org/10.1002/ffj.976
35. Polunin, O.; Stainton, A. Flowers of the Himalaya. Oxford University Press: New Delhi, 1984, 580 p.
36. Tabaca, N.; Demirci, F.; Demirci, B.; Wedge, D.E.; Başer, K.H.C. Composition, enantiomeric distribution and antimicrobial activity of *Tanacetum argenteum* subsp. *flabellifolium* essential oil. Journal of Pharmaceutical and Biomedical Analysis, 2007, 45, pp. 714-719. DOI: https://doi.org/10.1016/j.jpba.2007.08.006
37. Thomas, O.O. Chemical taxonomy and chemosystematics of the genus *Tanacetum*. Part 2. Phytochemistry of the leaf and flower oil of *Tananacetum corymbosum*. Fitoterapia, 1989, 60, pp. 225-228.
38. Zinicovscaia, I.; Ciocarlan, A.; Lupascu, L.; Aricu, A.; Dragalin, I.; Ciocarlan, N.; Yushin, N. Chemical analysis of *Tanacetum corymbosum* (L.) Sch. Bip. using neutron activation analysis. Journal of Radioanalytical and Nuclear Chemistry, 2019, 321, pp. 349-354. DOI: https://doi.org/10.1007/s10967-019-06590-x
39. Piqué, N.; Miñana-Galbis, D.; Merino, S.; Tomás, J.M. Virulence factors of *Erwinia amylovora*: A Review. International Journal of Molecular Sciences, 2015, 16(6), pp. 12836-12854. DOI: https://doi.org/10.3390/ijms160612836
40. Benada, M.; Boumaaza, B.; Boudalia, S.; Khaladi, O.; Guessas, B. Variability of aggressiveness and virulence of *Erwinia carotovora* subsp. *Carotovorum* causing the soft rot on potato tubers in the western of Algeria. International Journal of Plant Biology, 2018, 9(1), pp. 52-56. DOI: https://doi.org/10.4081/pb.2018.7568
41. Tamir-Ariel, D.; Navon, N.; Burdman, S. Identification of genes in *Xanthomonas campestris pv. vesicatoria* induced during its interaction with tomato. Journal of Bacteriology, 2007, 189(17), pp. 6359-6371. DOI: https://doi.org/10.1128/JB.00320-07
42. Jansen, B.J.M.; de Groot, A. The occurrence and biological activity of drimane sesquiterpenoids. Natural Product Reports, 1991, 8(3), pp. 309-318. DOI: https://doi.org/10.1039/NP9910800309
43. Chadwick, M.; Trewin, H.; Gawthrop, F.; Wagstaff, C. Sesquiterpenoids lactones: benefits to plants and people. International Journal of Molecular Sciences, 2013, 14(6), pp. 12780-12805. DOI: https://doi.org/10.3390/ijms140612780
44. Trombetta, D.; Castelli, F.; Sarpietro, M.G.; Venuti, V.; Cristani, M.; Daniele, C.; Saija, A.; Mazzanti, G.; Bisignano, G. Mechanisms of antibacterial action of three monoterpenes. Antimicrobial Agents and Chemotherapy, 2005, 49(6), pp. 2474-2478. DOI: https://doi.org/10.1128/AAC.49.6.2474-2478.2005