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Article

Variations in Chemical Compositions and Biological Activities of *Artemisia vulgaris* L. (Common Mugwort) Essential Oils at Different Growth Stages

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Abstract: The current study is aimed to investigate the essential oil (EO) yields, chemical compositions, antioxidant and antimicrobial activities of *Artemisia vulgaris* L. EOs were isolated before flowering, initial flowering and post-flowering growth stages. Before the flowering stage, major constituents of the EO were α -thujone (30.68%) and β -caryophyllene (22.05%). Parallel to plant development, sesquiterpene hydrocarbons (mainly β -caryophyllene) decreased and an increase was recorded in oxygenated monoterpenes, especially in α -thujone. These significant changes in the EO compositions caused various alterations in antioxidant and antimicrobial activities. Antioxidant activities of EOs were drastically decreased as the amount of β -caryophyllene dropped off from 22.05 to 7.92% and α -thujone increased from 30.68 to 47.41%. Spearman's correlation coefficients exhibited that α -thujone was quite weak against oxidative stress. In contrast to antioxidant activities, correlation analysis revealed that antimicrobial activities of EOs were enhanced with the increase of α -thujone.

Keywords: Artemisia vulgaris L.; Common mugwort; Essential oil; Biological activities; Growth periods.

Introduction

The genus Artemisia -one of the largest genera of Asteraceae- is mainly distributed in the northern temperate regions of the world. There are more than 500 species in the genus and 23 of them exist in Turkey while only 6 one of them grow naturally ^{1.4}. *Artemisia vulgaris* L. also known as mugwort, wormwood, felon herb and sailor's tobacco, is one of the important members of the genus and grows naturally widespread throughout of Turkey ²⁻⁴. The plant's genus name "Artemisia" derives from the Greek goddess and the epithet word "vulgaris" means common or usual ⁵. A. vulgaris is a branched shrub that is about 70-150 cm long. Rhizomes of the plant are light brown and could grow up to 1 cm in diameter. The dark green leaves are 5-10 cm and are characterized as pinnatisect or bipinnatisect. Red-brown or yellowish coloured flowers bloom through July to September and the fruit which is also known as "achenes" occurs after the flowering period ^{5,6}. *A. vulgaris* is a traditionally used medical plant and is usually utilised to treat various diseases such as gastric ulcers, inflammatory problems, diabetes, depression and stress disorders. Antioxidant, antibacterial, anthelmintic, antispasmodic, anticancer, antinociceptive and hepatoprotective biological properties have also been described ⁴⁻⁶.

A. vulgaris has a pale-yellow EO with a characteristic fragrance depending on the chemotype. The chemical composition of *A. vulgaris* EOs vary according to harvest time, climatic region and the plant parts distilled. Previous studies have identified a wide range of chemotypes including 1,8-cineole ^{4,7}, α -thujone ⁸, β -thujene ⁹, caryophyllene ¹⁰, caryophyllene oxide ¹¹, isoborneol ¹², borneol ¹³, germacrene D ¹, chrysanthenyl acetate ¹³⁻¹⁵, camphor ^{12,16}, α - β pinenes ^{17,18} and davanone B ¹⁹.

Plant physiology changes during growth, affecting biochemical and physical functions. The synthesis of terpenes and EO compounds are also expected to be influenced by these alterations. Although *A. vulgaris* is used as an important medicinal plant, variations in biochemical pathways and EO compositions parallel to plant growth have not been studied sufficiently. In this manuscript, we aimed to investigate the variations in chemical compositions of *A. vulgaris* EOs during different growth stages and their effects on antioxidant and antimicrobial activities.

Materials and methods

Methanol, n-hexanol, n-hexane, chloroform, Tween 40 and Tween 80 were purchased from Merck (Darmstadt, Germany). C_7-C_{40} alkane series, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, β -carotene, linoleic acid, α -pinene, 1,8-cineole, eugenol, β -caryophyllene and β -caryophyllene oxide (enantiomeric form) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Microorganisms and control substances were gently donated by Bezmialem Vakif University Medical Microbiology Department.

Collection of Plant Material

Cultivated A. vulgaris samples (approximately 8

kg) were collected from the same population at different growth stages -before flowering, initial flowering and post flowering- between August and October from Zeytinburnu Medicinal Plants Garden, İstanbul, Turkey (GPS coordinates 41°00'31.5"N 28°54'59.0"E). Plant materials were further identified by Prof. Dr. Murat Kartal (Bezmialem Vakif University, faculty of pharmacy, department of pharmacognosy). Voucher specimens were deposited in Zeytinburnu Medicinal Plants Garden herbarium with the numbers of BVUAV2101 to BVUAV2103.

Isolation of essential oil

The main stems of the plants were separated and rest of the aerial parts were air dried. 1000 grams of each plant materials were mechanically grounded before being distilled for 3 hours in triplicate using a Clevenger apparatus. The isolated EOs were dried with anhydrous Na_2SO_4 and samples were kept in amber vials at 4°C until analysis.

Analysis of essential oil

Analysis of EOs was performed on an Agilent GC-FID/MS system (Santa Clara, CA, USA). For this purpose, an Agilent 7890B GC-FID system coupled with an Agilent 5977E MS detector by using a capillary column splitter was employed. 1 μ L of sample EO solutions in *n*-hexane (10%, v/v) were injected with an Agilent G4513A autoinjector. Separations were performed with an HP-5MS column (30m, 0.25mm, 0.25µm) with the following temperature program; 70°C isothermal for 10 minutes and raised to 100°C with an increased rate of 2ºC/min. Later, temperature was increased to 230°C at a rate of 5°C/min and held isothermally for 4 minutes. Total analysis time was 55 minutes. Helium with a constant flow rate of 1.5 mL/min was used as a carrier gas. Split ratio was 1:50. Temperatures of other system components such as injector port, MSD transfer line, ion source, quadrupole and FID were maintained at 250°C, 250°C, 230°C, 150°C and 220°C respectively. FID dry air and H₂ flows were adjusted to 400 mL/min and 30 mL/min. Mass spectra was recorded between 45-450 m/z.

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Identification of compounds were performed by comparing their spectral data with NIST 11 Mass Spectral Library (NIST11/2011/EPA/NIH) and by co-injecting reference substances. C_{7} - C_{40} homologous alkane series were used to calculate retention indices and later compared with NIST online webbook data. Quantification was carried out utilizing an external standard approach based on calibration curves derived from GC-FID analyses of representative compounds.

Antioxidant activity assays

One of the most popular assays to estimate the radical scavenging capacities of EOs is DPPH free radical scavenging assay. It depends on the hydrogen or electron-donating capabilities of essential oil molecules. When antioxidant EOs react with DPPH radicals, the methanolic DPPH solutions change colour from purple to yellow which could be monitored spectrometrically. Another typical antioxidant test is the β-carotene/ linoleic acid assay (BCB), which is based on the radicals generated by linoleic acid autoxidation and discolouring the yellowish colour of β -carotene. A competitive reaction between β-carotene and antioxidant EOs could prevent discoloration when EO is added to the solution. The DPPH free radical scavenging activity and BCB assays were carried out using the same procedures as previously described ²⁰.

Antimicrobial activity assays

A. vulgaris EOs were tested against 3 Grampositive bacteria (*Bacillus cereus* ATCC 10876, *Clostridium perfringens* ATCC 13124, *Streptococcus pneumoniae* ATCC 6303), 3 Gram-negative bacteria (*Acinetobacter Iwoffii* ATCC 17925, *Escherichia coli* ATCC 10536, *Klebsiella pneumoniae* ATCC BAA-1706) and 2 yeast (*Candida krusei* ATCC 6258 and *Candida albicans* ATCC 18804) strains. The culturing of microorganism were performed according to methods previously described ²¹.

The Clinical and Laboratory Standards Institute's (CLSI) agar disc diffusion method and broth microdilution broth susceptibility assays ^{22,23} were conducted to determine the antimicrobial activities of the EOs as described earlier ²⁴. Ciprofloxacin (\geq 98%) and miconazole nitrate (\geq 99%) were used as positive controls for both tests against bacteria and yeasts respectively.

Statistical analysis

All analyses were performed in triplicate. Results were given as mean±standard deviation (SD). Comparisons were performed with oneway analysis of variance (ANOVA) followed by the Tukey post hoc test to determine significant differences between the means. Spearman's correlation coefficients were carried out to determine the relationships between the amounts of α -thujone and biological activities. Level of significance was taken α =0.05. Statistical analysis was performed using Graphpad Prism 8.0.1 (Graphpad Software, San Diego, CA, USA).

Results

Essential oil yields and compositions

Aerial parts of *A. vulgaris* yielded an average of 0.45% (gram/100 gram) EOs. Table 1 represents the stages of growth, harvesting times, essential oil yields and relative densities (g/cm³ at 20^oC) of *A. vulgaris* EOs. Values were presented as mean \pm SD.

Total of 44 compounds were detected and quantified in *A. vulgaris* EOs. Before the flowering stage, major constituents of the

 Table 1. Stage of growth, harvesting time, essential oil yields and relative densities of A. vulgaris L. essential oils

Stage of Growth	Harvesting Dates	% Yield	% Yield	Relative Density
		(mL/100 gram)	(g/100 gram)	
Before Flowering (BF)	10 August	0.52 ± 0.07	0.46 ± 0.06	0.8857 ± 0.0029
Initial Flowering (IF)	14 September	0.51 ± 0.06	0.46 ± 0.05	0.9102 ± 0.0013
Post Flowering (PF)	14 October	0.47 ± 0.08	0.42 ± 0.07	0.8916 ± 0.0017

EO were found to be α -thujone (30.68%) and β -caryophyllene (22.05%). Parallel to plant development caryophyllene decreased to 7.92% and α -thujone increased to 47.41%. With the onset of flowering, the EO chemotype shifted from α -thujone/caryophyllene to α -thujone. Chemical compositions according to plant growth stages were given in Table 2. Values were demonstrated as mean±SD.

Antioxidant activity

The antioxidant activities of *A. vulgaris* EOs were evaluated through DPPH and β -carotene/linoleic acid assays compared with ascorbic acid as reference. IC₅₀ values were calculated according to given formulas in a previous study²⁰. During the plant development, DPPH IC₅₀ values ascended from 36.82 to 43.31 µg/mL. BCB inhibition capability of EOs also decreased from

No	Compound	R.T	R.I ^L	R.I ^C	Concentration (%)			
					BF	IF	PF	
1	cis-Salvene	3.392	847	844	0.109±0.024	0.108±0.061	0.128±0.034	
2	2-Hexenal	3.440	854	856	1.046 ± 0.102	$0.835 {\pm} 0.624$	0.800 ± 0.380	
3	n-Hexanol#	3.685	867	867	0.445 ± 0.086	$0.230{\pm}0.097$	0.334 ± 0.128	
4	α-Pinene#	5.117	939	941	0.265 ± 0.033	$0.338 {\pm} 0.043$	$0.293{\pm}0.051$	
5	Sabinene	6.370	975	972	0.118 ± 0.041	$0.541 {\pm} 0.086$	0.632 ± 0.152	
6	β-Pinene	6.501	978	983	0.248 ± 0.076	$0.252{\pm}0.094$	$0.199{\pm}0.032$	
7	1-Octen-3-ol	6.700	986	988	0.658 ± 0.117	0.686 ± 0.228	$0.600 {\pm} 0.185$	
8	β-Myrcene	7.026	992	995	0.456 ± 0.086	1.044 ± 0.376	5.909 ± 1.284	
9	3-Octanol	7.361	994	997	0.126 ± 0.051	$0.149 {\pm} 0.032$	$0.155 {\pm} 0.027$	
10	p-Cymene	8.680	1021	1019	0.416 ± 0.033	$0.380{\pm}0.063$	$0.517 {\pm} 0.048$	
11	Limonene	8.878	1044	1051	$0.210{\pm}0.019$	N.D	N.D	
12	1,8-Cineole#	9.091	1046	1054	6.799 ± 0.893	9.635±1.428	8.678 ± 1.289	
13	γ-Terpinene	10.708	1064	1060	$0.180{\pm}0.102$	0.306 ± 0.047	$0.359 {\pm} 0.095$	
14	α-Thujone	14.268	1106	1108	30.686 ± 1.261	44.067 ± 2.586	47.410 ± 2.117	
15	β-Thujone	14.864	1119	1127	6.326 ± 1.042	8.878±1.146	9.109 ± 2.381	
16	cis-p-Mentha-2,8-dienol	15.224	1122	1129	0.269 ± 0.079	0.296 ± 0.035	0.192 ± 0.029	
17	trans-Pinocarveol	16.312	1140	1144	$0.565 {\pm} 0.061$	$0.617 {\pm} 0.047$	$0.554{\pm}0.081$	
18	Camphor	16.583	1145	1149	$0.690 {\pm} 0.077$	0.982 ± 0.236	1.120 ± 0.371	
19	Pinocarvone	17.841	1164	1166	0.452 ± 0.096	$0.449 {\pm} 0.107$	$0.334{\pm}0.097$	
20	Terpinene-4-ol	19.074	1172	1182	0.601 ± 0.102	1.003 ± 0.328	1.108 ± 0.472	
21	trans-Carveol	19.994	1217	1224	0.195 ± 0.063	0.236 ± 0.131	$0.296 {\pm} 0.043$	
22	cis-Carveol	22.321	1226	1231	1.584 ± 0.427	1.329 ± 0.472	$0.823 {\pm} 0.277$	
23	Carvone	23.713	1242	1239	0.754 ± 0.240	0.718 ± 0.341	0.518 ± 0.193	
24	Perillaldehyde	25.659	1279	1281	$0.317 {\pm} 0.084$	0.467 ± 0.159	$0.598 {\pm} 0.301$	
25	Eugenol#	30.083	1373	1370	0.311 ± 0.057	$0.348 {\pm} 0.062$	0.216 ± 0.103	
26	α-Copaene	30.467	1376	1375	0.249 ± 0.041	$0.117 {\pm} 0.038$	N.D	
27	β-Elemene	31.172	1388	1391	0.128 ± 0.017	N.D	N.D	
28	Caryophyllene#	32.188	1421	1430	22.059 ± 1.273	$12.054{\pm}1.086$	7.928±1.471	
29	α-Humulene	33.260	1454	1455	$3.594{\pm}1.102$	2.040 ± 0.946	1.402 ± 0.775	
30	trans-β-Farnesene	33.480	1455	1452	0.362 ± 0.067	$0.454{\pm}0.048$	$0.810{\pm}0.163$	
31	Germacrene D	34.180	1479	1482	8.217±1.902	4.381 ± 0.844	$2.563 {\pm} 1.007$	
32	Bicyclogermacrene	34.610	1494	1499	2.496 ± 0.871	1.562 ± 0.756	$1.085 {\pm} 0.388$	
33	α-Muurolene	34.735	1498	1502	0.192 ± 0.033	N.D	N.D	

Table 2. Compositions of A. vulgaris L. essential oils at different periods

N .7								
No	Compound	R.T	R.I ^L	R.I ^C	Concentration (%)			
					BF	IF	PF	
34	γ-Cadinene	35.127	1513	1517	0.141 ± 0.097	N.D	N.D	
35	δ-Cadinene	35.402	1516	1520	0.941 ± 0.263	$0.572 {\pm} 0.103$	$0.396 {\pm} 0.088$	
36	(Z)-Nerolidol	36.602	1560	1557	0.216 ± 0.047	$0.157 {\pm} 0.031$	$0.154{\pm}0.019$	
37	Germacrene D-4-ol	36.900	1568	1565	0.304 ± 0.073	$0.228 {\pm} 0.095$	0.145 ± 0.082	
38	Spathulenol	36.975	1571	1569	0.387 ± 0.156	$0.197{\pm}0.044$	N.D	
39	Caryophyllene oxide#	37.044	1574	1578	$0.694{\pm}0.102$	0.371 ± 0.157	$0.633 {\pm} 0.089$	
40	Alloaromadendrene	37.121	1595	1597	$0.292{\pm}0.071$	N.D	N.D	
	oxide							
43	tau-Cadinol	38.601	1626	1632	$0.745 {\pm} 0.094$	$0.434{\pm}0.173$	0.220 ± 0.039	
44	α-Cadinol	38.919	1641	1643	1.177 ± 0.383	0.491 ± 0.272	$0.307 {\pm} 0.089$	
Tota	al Identified				96.020 96.952 96.525		96.525	
Phy	Phytochemical Classes							
Oxygenated Hydrocarbons (2, 3, 7, 9)				2.275	1.900	1.889		
Mo	noterpene Hydrocarbons (1, 4-6, 8,	, 10, 11	, 13)	2.002	2.969	8.037	
Oxygenated Monoterpenes (12, 14-24)				49.238	68.677	70.740		
Sesquiterpene Hydrocarbons (26-35)		38.379	21.18	14.184				
Oxygenated Sesquiterpenes (36-40, 43, 44)		3.815	1.878	1.459				
Phenylpropanoids (25) 0.311 0.348 0.216						0.216		
#; C	#; Co-injected authentic samples of reference substances for identification and quantitation, R.T; Retention							
time	time (min), R.I ^L ; Retention indices derived from literature and NIST database, R.I ^c ; Calculated retention							

table 2. (continued).

67.7% to 53.1% parallel to plant development. Results were presented as mean±SD and shown in Table 3.

Antimicrobial activity

indices, N.D; Not detected

In contrast to antioxidant capabilities, overall antimicrobial activities of EOs were enhanced during plant development in both disc diffusion and broth microdilution assays. The antimicrobial activities of *A. vulgaris* EOs over the studied organisms were listed in Table 4. The inhibition zone diameters (IZD) were measured with a digital caliper as millimeters (mean \pm SD) including the disc diameters. Minimum inhibitory concentrations (MIC) were given in mg/mL for EOs and µg/mL for positive controls.

Correlation tests

Spearman correlation coefficients were carried out to determine the relationships between the amounts of α -thujone in EOs and the biological activities. Correlation analysis demonstrated that there were negative relationships between α -thujone and the antioxidant activites. On the other hand, strong to moderate correlations were found in antimicrobial assays. Correlations were shown in Table 5.

Discussion

Essential oil yields of *A. vulgaris* at different periods were found to be similar with no significant differences by statistical means (Table 1). The overall yield was determined to be approximately 0.5%. Our results were similar to an earlier study from the East Anatolian region of Turkey which reported 0.5% yield with β -thujene chemotype⁹. Another study from South India obtained 1.3% EO yield with α -thujone chemotype ⁸. Nepal originated α -thujone chemotype *A. vulgaris* was reported to yield 0.8% EO ²⁵. The presence of so many chemotypes limits the literature data on EO yields of α -thujone chemotype *A. vulgaris*.

Total of 44 compounds were identified and quantified in *A. vulgaris* EOs (Table 2). The EOs

Essential Oils and Controls	DPPH IC ₅₀ (µg/mL)	BCB (%)				
Before Flowering	36.82±1.54 ^{ab}	67.6±2.5°				
Initial Flowering	40.67±0.98ª	59.7±1.6°				
Post Flowering	42.31±1.86 ^b	53.1±2.2°				
Ascorbic Acid	5.82 ± 0.96	93±2.4				
Antioxidant activities of essential oils had statistically significance						
differences within the growth stages. The values which are followed by						
the same letter in the same column were significantly different at $n < 0.05$						

 Table 3. Antioxidant activities of A. vulgaris L. essential oils

Table 4. Antimicrobial activities of A. vulgaris L. essential oils

Organisms	Before F	owering	Initial Flowering		Post Flo	Post Flowering		Positive Control	
	IZD	MIC	IZD	MIC	IZD	MIC	IZD	MIC	
A. lwoffii	17±1.6	0.3	21±0.9	0.1	20±1.8	0.1	31±2.3	1.0	
B. cereus	11±1.1	0.5	13±1.3	0.2	15±1.6	0.3	36±3.1	0.25	
C. perfringens	16±0.6	0.3	20±1.7	0.1	19±1.4	0.1	27±2.4	1.0	
E. coli	12±1.3	0.5	14±1.5	0.2	15±1.9	0.2	44±3.7	0.1	
K. pneumoniae	10 ± 1.5	0.7	12±1.2	0.4	12 ± 1.8	0.5	32±3.1	0.5	
S. pneumoniae	19±1.7	0.2	20±1.8	0.1	20±2.3	0.1	35 ± 2.8	0.5	
C. krusei	15±0.9	0.3	18±1.3	0.2	17±1.1	0.2	27±2.3	0.5	
C. albicans	13±2.1	0.4	16±2.3	0.3	16±1.6	0.3	29±3.2	1.0	
Ciprofloxacin and miconazole nitrate were used as positive controls for bacteria and yeasts respectively.									
Minimum inhibitory concentrations (MIC) were given in mg/mL for essential oils and μ g/mL for positive controls									

were dominated by oxygenated monoterpenes at all growth periods. The major constituents of the EOs in pre-flowering stage (August) were α -thujone (30.68%) and β -caryophyllene (22.05%). This result was similar to a previous study conducted in USA that demonstrated 24.3% α-thujone and 16.5% β-caryophyllene with α -thujone/ β -caryophyllene chemotype EO. The study also mentioned a β-caryophyllene/ santolinatriene chemotype obtained from a different population separated by a distance of a few meters from α-thujone/β-caryophyllene chemotype ¹⁵. This could indicate different chemotypes of A. vulgaris might exist in the same location even side by side. In flowering stage, while the flowers were just blooming (September), composition of the EO changed drastically. a-thujone increased to 44.06% and β -caryophyllene decreased to 12.05% resulting in an α -thujone chemotype. Minor oxygenated monoterpene compounds such as β -thujone and 1,8-cineole were also increased during the flowering stage. The amount of total sesquiterpenes -both hydrocarbons and oxygenateddecreased together with β -caryophyllene. In October, post-flowering stage while the flowers were bloomed already, a-thujone performed a small increment to 47.41% together with β -thujone (9.10%) while total sesquiterpenes kept dropping off. In the final stage of development, A. vulgaris EO was still α -thujone chemotype. The changes in EO chemistry could be explained by the alteration of enzymatic pathways. A possible mechanism might be the suppression of farnesyl pyrophosphate synthase which catalyses the condensation of isopentenyl pyrophosphate to geranyl pyrophosphate (an intermediate in the synthesis of monoterpenoids) to form farnesyl pyrophosphate which is known as the intermediate for sesquiterpenoid synthesis.

Activity	α-Thujone			
	r _s	p-value		
DPPH (IC ₅₀)	0.95	< 0.001		
BCB (%)	-1.00	< 0.001		
IZD A. lwoffii	0.93	< 0.001		
IZD B. cereus	0.56	0.121		
IZD C. perfringens	0.91	0.001		
IZD E. coli	0.80	0.014		
IZD K. pneumoniae	0.76	0.022		
IZD S. pneumoniae	0.52	0.150		
IZD C. krusei	0.91	0.001		
IZD C. albicans	0.76	0.022		
MIC A. lwoffii	-0.82	0.024		
MIC B. cereus	-0.94	0.001		
MIC C. perfringens	-0.82	0.024		
MIC E. coli	-0.82	0.024		
MIC K. pneumoniae	-0.94	0.001		
MIC S. pneumoniae	-0.82	0.024		
MIC C. krusei	-0.82	0.024		
MIC C. albicans	-0.82	0.024		

Table 5. Spearman's correlation coefficient of α-thujone

A. vulgaris EOs exhibited lower antioxidant activities (Table 3) compared to ascorbic acid as reference both in DPPH and β -carotene/ linoleic acid assays. The IC_{50} values for DPPH were increased to 42.3 from 36.8 µg/mL and BCB inhibitions were decreased to 67.6 to 53.1% during the studied growth periods. The oxygenated monoterpenes in A. vulgaris EOs were highly in ketone form. In contrast to the rising in oxygenated monoterpenes, antioxidant activities were found to be lower. These ketone compounds might be the reason for the gradually decreasing antioxidant activity as they simply lack proton donating hydroxyl groups. A similar conclusion was made in a previous study with Artemisia herba-alba EO²⁶. Correlation analysis (Table 5) revealed that the amount of α -thujone had a strong positive relation with DPPH IC₅₀ test results ($r_s = 0.95$ and p = < 0.001) and a negative relationship with BCB inhibition ($r_s = -1.00$ and p = < 0.001). Overall, there was a strong negative correlation between the amount of α -thujone and antioxidant activities.

A. vulgaris EOs performed moderate antimicrobial activities (Table 4). Although it is not appropriate to attribute the antimicrobial activity of EOs into a single compound, it could be concluded that the antibacterial activity of A. vulgaris EOs escalated as the amount of α -thujone increased. Correlation analysis demonstrated that there are moderate to strong positive relationships between the amount of α -thujone and the antimicrobial activities depending on the tested species.

Various extracts and EOs obtained from Artemisia species were also evaluated for their biopesticide abilities such as fungicide, insecticide, nematicide and herbicide ²⁷. Dense fungicide activities were associated with high amount of thujones in EOs 28,29. Essential oil compounds which disrupt GABA synapses were previously cited as insecticide acting through neurotoxic effects $^{\rm 30}$ and $\alpha\text{-thujone}$ was known to be a toxic compound modulating GABA (γ -Aminobutyric acid) type A receptors ³¹. Essential oil of Artemisia herba-alba which contains high amount of thujones performed significant nematocidal activity ³². Artemisia nilagirica EO presented prominent nematocidal activity which contains α -thujone as the major compound ³³. α -Thujone rich EOS derived from Artemisia absinthium, Artemisia fragrans, Artemisia sieversiana demonstrated notable herbicide activities 34-36. Artemisia vulgaris EO, rich in a-thujone, might be expected to possess the same biopesticidal effects.

Conclusions

A. vulgaris is traditionally used for medicinal purposes. Most of its medicinal effects are attributed to its EO. It is also known to have various chemotypes. Literature data on α -thujone chemotype *A. vulgaris* EO is limited. It would be appropriate to collect α -thujone chemotype *A. vulgaris* during and after the flowering stages if it is intended to be used due to its antimicrobial activity. Further studies are also required to determine the possible medical effects of thujone-rich *A. vulgaris* essential oil and its usability as a potent biopesticide.

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Declaration of interest

The authors declare no conflicts of interest in this work.

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