Chemical Diversity and Biological Potential of *Tanacetum praeteritum* subsp. *praeteritum* Essential Oils

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Abstract: Two samples of *Tanacetum praeteritum* (Horwood) Heywood subsp. *praeteritum* (Horwood) were collected in flowering period and subjected separately to hydrodistillation to yield the essential oils (A and B). The oils were investigated for chemical composition with GC-FID and GC/MS techniques and evaluated against acetylcholinesterase and α-amylase enzymes and free radicals (DPPH$^{\bullet}$ and ABTS$^{\bullet+}$) using microtiter plate assays. Both oils were characterized with high abundance of oxygenated monoterpenes. The oils were distinguished by the main constituents, namely, camphor (37.6%), 1,8-cineole (19.5%) and terpinen-4-ol (9.3%) were found as the major constituents in the oil A, while α-thujone (79.4%) and β-thujone (8.5%) were detected in the oil B. The oils demonstrated significant inhibitory (80% and 60.0%) potentials on acetylcholinesterase (an IC_{50} of 0.74 mg/mL and an IC_{50} of 1.78 mg/mL, respectively) which is involved in Alzheimer's disease. With respect to antidiabetic activity, the oils demonstrated significant inhibiting potential on porcine pancreatic α-amylase (an IC_{50} of 1.02 mg/mL and an IC_{50} of 0.89 mg/mL) in I_2/KI assay. The oils demonstrated weak free radical scavenging activity against DPPH radicals and moderate activity (0.23 mM and 0.15 mM) against ABTS$^{+\bullet}$ in TEAC assay.

Keywords: *Tanacetum*; essential oil; chemical composition; GC/MS; activity.

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INTRODUCTION

The genus *Tanacetum* is one of large genera of Asteraceae family. The genus is represented by nearly 200 species around the world. In Turkey, the genus *Tanacetum* encompasses 60 taxa (1).

Different type extracts (solvent extracts, essential oils and fractions) and pure compounds isolated from *Tanacetum* species have been reported for a range of biological activities. Namely, antibacterial, antioxidant (2), anthelmintic (3), repellent, insecticidal (4), cytotoxic and acetylcholinesterase inhibitory (5), and antidiabetic (6). Flavonoids, mono-, di- and sesquiterpenes, and sesquiterpene lactones have been found to be responsible for biological activities of *Tanacetum* species (7-11).

Nowadays, there is increasing demand for effective and safe natural products with antioxidant, antiacetylcholinesterase and hypoglycemic properties. The efficacy of known synthetic antioxidants (12), antiacetylcholinesterase agents (13), and hypoglycemic products (14, 15) is debatable. The plants reputed for their neurodegenerative healing and antidiabetic effects should be verified either experimentally or clinically. However, evidenced-based therapeutic usage of many plants is scarce.

T. praeteritum subsp. *praeteritum* has earlier been reported for flavonoids (16), sesquiterpene lactones (17), eudesmane-type sesquiterpenes (18) essential oil (19). However, there is no information about its biological activity. The present work is the first investigation of *T. praeteritum* subsp. *praeteritum* essential oil for antioxidant, antiacetylcholinesterase and antidiabetic potential.

MATERIALS AND METHODS

Chemicals

Hydrochloric acid, *n*-hexane, dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Germany), anhydrous sodium sulfate (Fluka, Germany), iodine (ACS reagent), potassium iodide (Saint Louis, USA), methanol (Sigma-Aldrich, Poland), potassium persulfate (Sigma-Aldrich, Saint Louis, USA), sodium phosphate, and disodium phosphate were of analytical grade. A C_8–C_{40} *n*-alkane standard solution was purchased from Fluka (Buchs, Switzerland). Gallic acid (GA), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), soluble starch, acarbose, α-amylase from porcine pancreas (Type VI-B, EC 3.2.1.1), tris(hydroxymethyl) aminomethane (ACS reagent), acetylcholinesterase (AChE) from *Electrophorus electricus* (Type VI-S), bovine serum albumin (BSA), acetylthiocholine iodide (ATCI), 5,5′-dithiobis(2-

nitrobenzoic acid) (DTNB), galanthamine from *Lycoris* sp. were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Instrumentation

Agilent 5975 GC-MSD system (Agilent, USA; SEM Ltd., Istanbul, Turkey) was equipped with the HP-Innowax FSC column (60 m × 0.25 mm id with 0.25 μm film thickness, Agilent, USA). The GC-FID analysis was carried out with capillary GC using an Agilent 6890N GC system (SEM Ltd., Istanbul, Turkey). Microtiter plate assays were performed with Biotek Powerwave XS microplate reader. Ultrapure water (0.05 μS/cm) was obtained from a Direct-Q® Water Purification System (Germany).

Plant Material

Plant materials were two samples (A and B) of *T. praeteritum* subsp. *praeteritum* collected in period of flowering in Karçukuru Yaylasi in Antalya province of Turkey, on June, 2015. The plant material was dried under the shade. Botanical identification was performed by Dr. M. Arslan. Voucher specimens are deposited at the Herbarium of the Forest Regional Department.

Hydrodistillation of Essential Oil

Aerial parts of two *T. praeteritum* ssp*. praeteritum* samples (A and B) were separately subjected to hydrodistillation (3 h) in a Clevenger type apparatus (Eu.Ph.) to yield essential oils (20).

Gas-Chromatography - Mass Spectrometry (GC/MS)

The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system (Agilent, USA; SEM Ltd., Istanbul, Turkey). HP-Innowax FSC column (60 m × 0.25 mm, 0.25 μm film thickness, Agilent, USA) was used with a helium carrier gas at 0.8 mL/min. GC's oven temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4 °C/min, kept constant for 10 min at 220 °C, and then programmed to increase at a rate of 1 °C/min to 240 °C. The oils were analyzed with a split ratio of 40:1. The injector temperature was 250 °C. Mass spectra were taken at 70 eV and the mass range was from m/z 35 to 450.

Gas Chromatography – Flame Ionization Detection (GC-FID)

The GC-FID analysis was carried out with capillary GC using an Agilent 6890N GC system (SEM Ltd., Istanbul, Turkey). Flame ionization detector (FID) temperature was set at 300 °C in order to obtain the same elution order with GC/MS. Simultaneous injection was performed using the same column and appropriate operational conditions.

Identification and Quantification of Compounds

Identification of the volatile constituents was achieved as reported previously (21). Briefly, identification of the individual compounds was based on the following: (i) comparison of the

GC/MS Relative Retention Indices (RRI) of the compounds on polar column determined relative to the retention times of a series of *n*-alkanes (C_8-C_{40}), with those of authentic compounds or literature data; (ii) computer matching with commercial mass spectral libraries: MassFinder software 4.0 (22), Adams Library (23), Wiley GC/MS Library (Wiley, New York, USA) and NIST Library, and comparison of the recorded spectra with literature data Confirmation was also achieved using the in-house "Başer's Library of Essential Oil Constituents" database, obtained from chromatographic runs of pure compounds performed with the same equipment and conditions (24).

Determination of Anti-Acetylcholinesterase Activity (Ellman's Assay)

The essential oils were tested for inhibition of acetylcholinesterase (AChE) using Ellman's method as previously reported (25) with slight modification. Three buffers were used: (A) 50 mM Tris-HCl (pH=8.0, in ultrapure water); (B) 0.1% BSA in buffer A; (C) 0.1 M NaCl and 0.02 M $MgCl_2 \cdot 6H_2O$ in buffer A. The oils were previously dissolved in DMSO (20% in buffer). In the 96-well flat bottom plates, 25 μL of the sample (EO or standard), 50 μL of buffer B and 25 μL of AChE (0.22 U/mL in buffer A) solution were pipetted with 8-channel automatic pipette (Eppendorf Research® plus, Germany) and incubated for 15 min at 25 °C. Then, 125 μL of Ellman's reagent DTNB (3.0 mM in buffer C) and 25 μL substrate ATCI (15 mM, in ultrapure water) were added. Hydrolysis of ATCI was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines, catalyzed by enzymes at 412 nm utilizing a 96-well microplate reader (Biotek Powerwave XS, USA). The mixture allowed to stand 15 min at 25 °C and the absorbance was recorded at 412 nm. Similarly, a blank (for eliminating the colors of the samples) was prepared by adding sample solution to all reaction reagents and 25 μL buffer instead of enzyme. The control wells contained all the reagents without the sample (the solvents of the samples instead were added). Galanthamine hydrobromide (0.1 mg/mL) was used as positive control. The percentage inhibition was calculated according to Equation 1:

$$\%Inh = \left[\frac{(Abs_{control} - Abs_{control\ blank}) - (Abs_{sample} - Abs_{sample\ blank})}{Abs_{control} - Abs_{control\ blank}}\right] \times 100 \qquad \text{(Eq. 1)}$$

where $Abs_{control}$ and $Abs_{control\ blank}$ are the absorbance of the control and its blank, Abs_{sample} and $Abs_{sample\ blank}$ are the absorbance of the sample and its blank.

Determination of Anti-α-Amylase Activity

The activity of α-amylase under effect of *T. praeteritum* ssp. *praeteritum* essential oils was measured using Caraway-Somogyi iodine/potassium iodide (I_2/KI) method (26) with slight modifications. The substrate solution (0.05%) was prepared by dissolving of soluble potato starch (10 mg) in 20 mL ultrapure water then boiling for 10 min and cooling to room temperature before use. As a positive control experiment, acarbose (0.01-0.1 mg/mL in buffer) was used. In the experiment, 20 mM sodium phosphate buffer (pH 6.9) was pipetted in the 96-well flat bottom plates with multichannel automatic pipette (Eppendorf Research® plus, Germany), then 25 μL

of sample solution and 50 μL of α-amylase (0.8 U/mL in buffer) were added and incubated for 10 min at 37 °C. After incubation, 50 μL of substrate solution was added to the mixture. The mixture was subjected to a second incubation for 10 min at 37 °C. The reaction was stopped by addition of 25 μL of HCl solution (1 M). Finally, 100 μL of I_2/KI reagent was added to the wells. The sample blanks contained all reaction reagents and 50 μL of buffer instead of enzyme. The control wells contained all reaction reagents and 25 μL of solvent (instead of the sample solution). The absorbance values were recorded for the sample and blank at 630 nm. The percentage inhibition of the α-amylase activity (Inh%) was calculated according to Equation 1.

Antioxidant Activity

Free radical scavenging activity (DPPH assay)

The hydrogen atoms or electrons donation ability of the oils were evaluated according to bleaching of purple-colored DPPH stable radicals. The effect of *T. praeteritum* ssp*. praeteritum* essential oils on DPPH free radicals was determined using a method of Brand-Williams (27) with slight modifications. The DPPH solution (0.08 mg/mL, in methanol) was freshly prepared daily, kept in the dark at 4 °C between the measurements. The solutions of the essential oils (30 mg/mL) and gallic acid (0.1 mg/mL) were prepared in methanol. 100 μL of the sample (oil or standard) solution and 100 μL of DPPH solution were pipetted by multichannel automatic pipette (Eppendorf Research® plus, Germany) into 96-flat bottom well plate cells and allowed to stand in the dark for 30 min. The control well contained 100 μL methanol (instead of the sample) mixed with 100 μL of DPPH. The decrease in the absorbance was recorded at 517 nm. Gallic acid (standard) was used as positive control. Experiments were performed in triplicate. The free radical scavenging activity of the samples was expressed as percentage of inhibition calculated according to Equation 2:

$$\%\ Inh = \left(\frac{Abs_{control} - Abs_{sample}}{Abs_{control}}\right) \times 100 \qquad \text{(Eq. 2)}$$

where, $Abs_{control}$ is the absorbance of the control (containing all reagents except the test compound), Abs_{sample} is the absorbance of the sample with added DPPH. The IC_{50} values were obtained by plotting the DPPH scavenging percentage of each sample against the sample concentration.

Trolox-equivalent antioxidant capacity (TEAC assay)

$ABTS^{\bullet+}$ free radical cation scavenging activity of the samples were tested according to the procedure described by Re *et al*. (28) with slight modifications. 7 mM ABTS and 2.5 mM $K_2S_2O_8$ dissolved in 10 mL of ultrapure water were allowed to stand in dark for 16 h at room temperature to create $ABTS^{\bullet+}$ free radical cation. Prior to the assay, $ABTS^{\bullet+}$ solution was diluted with absolute ethanol to an absorbance between 0.7-0.8 at 734 nm. The solutions of the essential oils (5 mg/mL), and Trolox (standard, 3.0; 2.0; 1.0; 0.5; 0.25; 0.125 mM) were prepared in MeOH. 10 μL of the sample solution was mixed with 990 μL of $ABTS^{\bullet+}$ solution. 10 μL of MeOH instead of sample or standard mixed with $ABTS^{\bullet+}$ solution was used as control. Gallic acid (0.1 mg/mL)

was used as the positive control. Decrease in the absorbance after 30 minutes of incubation was recorded at 734 nm to get a linear Trolox equation. $ABTS^{\bullet+}$ scavenging activity of the samples was expressed as Trolox equivalent antioxidant capacity and calculated using linear equation obtained for Trolox (y =23,224x - 1,7141, r^2 = 0.9989) (Figure 1).

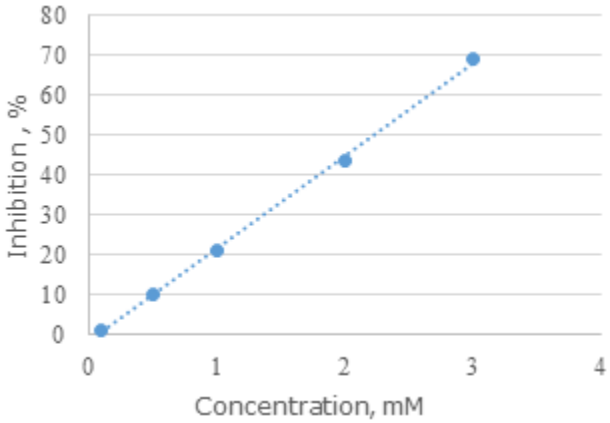

Figure 1. Calibration curve obtained for Trolox.

Statistical Analysis of Data

Data obtained from antioxidant and enzyme inhibition experiments were expressed as mean standard error (±SEM). IC_{50} values were estimated using a nonlinear regression algorithm. Data were analyzed using the *SigmaPlot* software (Version 12.0).

RESULTS AND DISCUSSION

In course of our ongoing studies on biodiversity of Turkish essential oil bearing plants, the composition of *Tanacetum praeteritum* ssp. *praeteritum* essential oils (A and B) from two localities and their biological activities were comparatively studied. The oils were hydrodistilled from aerial parts of the plants and phytochemically investigated with GC-FID and GC/MS techniques. The essential oils of *T. praeteritum* ssp. *praeteritum* obtained by hydrodistillation method were yellow with a distinct odor. The oils yields obtained after 3 h were 0.18% and 0.24% (w/w) for the oils A and B, respectively.

GC-FID and GC/MS analyses of the oils A and B resulted with 60 and 53 compounds respectively which belong to monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes. Distribution of the major compound groups detected in the oils A and B of *T. praeteritum* ssp. *praeteritum* is presented on Figure 2. The list of detected compounds with their relative retention indices, relative percentages and method of identification is given in Table 1 in order of their elution on the HP-Innowax FSC column. Gas-chromatographic profiles of *T. praeteritum* ssp. *praeteritum* oils is presented on Figure 3. Gas chromatographic study revealed distinguish difference between the oils A and B. The oils A and

B were characterized by predominance of the oxygenated monoterpenes (79.5% and 95.8%, respectively). However, the main constituents were different. Namely, camphor (37.6%), 1,8-cineole (19.5%) and terpinen-4-ol (9.3%) were found as the major constituents in the oil A, while α-thujone (79.4%) and β-thujone (8.5%) were detected in the oil B. The monoterpene hydrocarbons constituted 17.1% in the oil A with *p*-cymene (8.6%) and camphene (6.2%) as the main representatives. However, the oil B was characterized by scarce amount (2.1%) of this group. The sesquiterpenes were detected in low amounts in both of the oils (1.5% and 0.7%).

Table 1. Chemical compositions of *Tanacetum praeteritum* ssp. *praeteritum* essential oils.

No	RRI[a)]	RRI[b)]	Compound	%[b)]		ID
				A	B	Method
1.	1014	1017(29)	Tricyclene	0.3	-	c),d),e)
2.	1032	1029(29)	α-Pinene	0.6	0.3	c),d),e)
3.	1034	1029(29)	α-Thujene	0.8	0.2	c),d),e)
4.	1076	1074(29)	Camphene	**6.2**	0.1	c),d),e)
5.	1118	1116(29)	β-Pinene	0.3	0.2	c),d),e)
6.	1132	1132(30)	Sabinene	0.2	0.1	c),d),e)
7.	1174	1161(29)	Myrcene	-	t	c),d),e)
8.	1188	1180(29)	α-Terpinene	-	0.2	c),d),e)
9.	1195	1193(31)	Dehydro-1,8-cineole	0.1	t	c),d),e)
10.	1203	1204(29)	Limonene	-	t	c),d),e)
11.	1213	1211(29)	1,8-Cineole	**19.5**	4.3	c),d),e)
12.	1244		2-Pentyl furan	t	-	c),d),e)
13.	1255	1255(32)	γ-Terpinene	0.1	0.4	c),d),e)
14.	1280	1280(32)	*p*-Cymene	**8.6**	0.5	c),d),e)
15.	1285	1304(33)	Isoamyl isovalerate	t	-	c),d),e)
16.	1290	1290(32)	Terpinolene	t	0.1	c),d),e)
17.	1296	1283(34)	Octanal	t	-	c),d),e)
18.	1437	1428(35)	α-Thujone	-	**79.4**	c),d),e)
19.	1451	1446(35)	β-Thujone	-	**8.5**	c),d),e)
20.	1474	1474(32)	*trans*-Sabinene hydrate	2.4	0.3	c),d),e)
21.	1478		Norbornyl acetate	t	-	d),e)
22.	1482		(*Z*)-3-Hexenyl-2-methyl butyrate	-	t	d),e)
23.	1532	1532(30)	Camphor	**37.6**	0.7	c),d),e)
24.	1542	1564(36)	*cis*-Sabinene hydrate acetate	1.6	-	c),d),e)
25.	1553	1553(30)	Linalool	-	t	c),d),e)
26.	1556	1555(30)	*cis*-Sabinene hydrate	-	0.2	c),d),e)
27.	1558	1548(36)	Linalyl acetate	-	t	c),d),e)
28.	1568		1-Methyl-4-acetylcyclohex-1-ene*	0.4	0.4	e)

No	RRI[a)]	RRI[b)]	Compound	%[b)]		ID Method
				A	B	
29.	1575	1578(33)	*trans*-*p*-Menth-2-en-1-ol	0.9	0.1	c),d),e)
30.	1582	1561(30)	*cis*-Chrysanthenyl acetate	0.6	-	c),d),e)
31.	1586	1587(37)	Pinocarvone	t	t	c),d),e)
32.	1590	1597(32)	Bornyl acetate	0.1	-	c),d),e)
33.	1611	1611(32)	Terpinen-4-ol	**9.3**	1.0	c),d),e)
34.	1612	1612(37)	β-Caryophyllene	t	t	c),d),e)
35.	1630		Terpinen-4-yl acetate	0.3	-	d),e)
36.	1640	1645(33)	*cis*-*p*-Menth-2-en-1-ol	0.6	-	c),d),e)
37.	1670	1671(33)	*trans*-Pinocarveol	0.1	-	c),d),e)
38.	1682	1687(33)	δ-Terpineol	0.2	0.1	c),d),e)
39.	1683	1685(30)	*trans*-Verbenol	-	0.1	c),d),e)
40.	1685	1675(36)	*trans*-Piperitol	0.3	t	c),d),e)
41.	1694		Drima-7,9(11)-diene	-	0.1	d),e)
42.	1702		Selina-4(14),7-diene	-	t	d),e)
43.	1706	1706(37)	α-Terpineol	1.7	0.5	c),d),e)
44.	1719	1719(37)	Borneol	0.9	0.2	c),d),e)
45.	1720	1720(38)	*trans*-Sabinol	-	0.3	d),e)
46.	1726	1722(32)	Germacrene D	-	t	c),d),e)
47.	1729	1729(39)	*cis*-1,2-Epoxy-terpin-4-ol	0.3	-	d),e)
48.	1742	1741(30)	β-Selinene	-	t	c),d),e)
49.	1744	1735(33)	α-Selinene	-	t	c),d),e)
50.	1748	1744(33)	Piperitone	t	-	c),d),e)
51.	1751	1750(33)	Carvone	0.1	-	c),d),e)
52.	1755	1747(33)	Bicyclogermacrene	-	0.1	c),d),e)
53.	1758	1757(33)	*cis*-Piperitol	0.3	t	c),d),e)
54.	1764	1764(40)	*cis*-Chrysanthenol	1.7	-	c),d),e)
55.	1782	1772(41)	*cis*-Carvyl acetate	0.1	-	c),d),e)
56.	1802	1800(33)	Cumin aldehyde	0.1	-	c),d),e)
57.	1804	1807(33)	Myrtenol	t	-	c),d),e)
58.	1805	1793(40)	α-Campholene alcohol	t	t	c),d),e)
59.	1845	1845(30)	*trans*-Carveol	0.1	-	c),d),e)
60.	1864	1856(30)	*p*-Cymen-8-ol	0.2	-	c),d),e)
61.	1882	1880(37)	*cis*-Carveol	0.3	-	c),d),e)
62.	1969	1959(30)	*cis*-Jasmone	-	t	d),e)
63.	1985	1965(42)	2-Phenylethyl-2-methylbutyrate	-	t	c),d),e)
64.	2008	2008(32)	Caryophyllene oxide	0.2	0.2	c),d),e)
65.	2037	2037(32)	Salvial-4(14)-en-1-one	t	-	c),d),e)
66.	2050	2042(43)	(*E*)-Nerolidol	0.3	-	c),d),e)
67.	2056		13-Tetradecanolide	0.1	t	d),e)

No	RRI[a)]	RRI[b)]	Compound	%[b)]		ID Method
				A	B	
68.	2063	2041(44)	*p*-Mentha-1,4-dien-7-ol	-	t	d),e)
69.	2084	2084(45)	Octanoic acid	t	-	c),d),e)
70.	2113	2114(46)	Cumin alcohol	t	t	c),d),e)
71.	2144	2136(33)	Spathulenol	0.5	0.3	c),d),e)
72.	2184	2184(47)	*cis*-*p*-Menth-3-en-1,2-diol	0.1	-	c),d),e)
73.	2192	2173(48)	Nonanoic acid	0.1	-	c),d),e)
74.	2198	2187(35)	Thymol	0.1	-	c),d),e)
75.	2232	2236(37)	α-Bisabolol	t	t	c),d),e)
76.	2239	2239(37)	Carvacrol	-	0.1	c),d),e)
77.	2247	2247(32)	*trans*-α-Bergamotol	-	t	c),d),e)
78.	2260	2260(40)	15-Hexadecanolide	0.3	0.2	d),e)
79.	2273	2273(49)	Selin-11-en-4α-ol	0.3	-	c),d),e)
80.	2324	2324(50)	Caryophylla-2(12),6(13)-dien-5α-ol	0.1	-	c),d),e)
81.	2365	2349(51)	(*Z*)-Methyl jasmonate	t	t	c),d),e)
82.	2931	2931(32)	Hexadecanoic acid	t	t	c),d),e)
			Total	**99.0**	**99.2**	

[a)] Relative Retention Indices calculated against *n*-alkanes (C_8-C_{40}) on HP-Innowax column; [b)]% calculated from FID data; [c)] Identification based on retention index of genuine compounds on the HP-Innowax column; [d)] Identification on the basis of computer matching of the mass spectra from Başer; [e)] Tentative identified on the basis of computer matching of the mass spectra from Adams, MassFinder, Wiley, and NIST libraries; t Trace (< 0.1%).

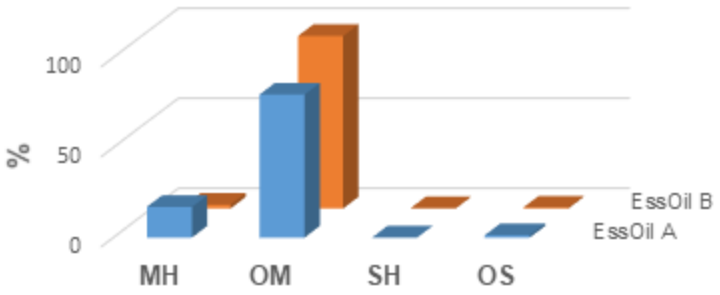

Figure 2. Distribution of the major compound groups in the oils A and B of *Tanacetum praeteritum* ssp. *praeteritum*. MH, monoterpene hydrocarbons; OM, oxygenated monoterpenes; SH, sesquiterpene hydrocarbons; OS, oxygenated sesquiterpenes.

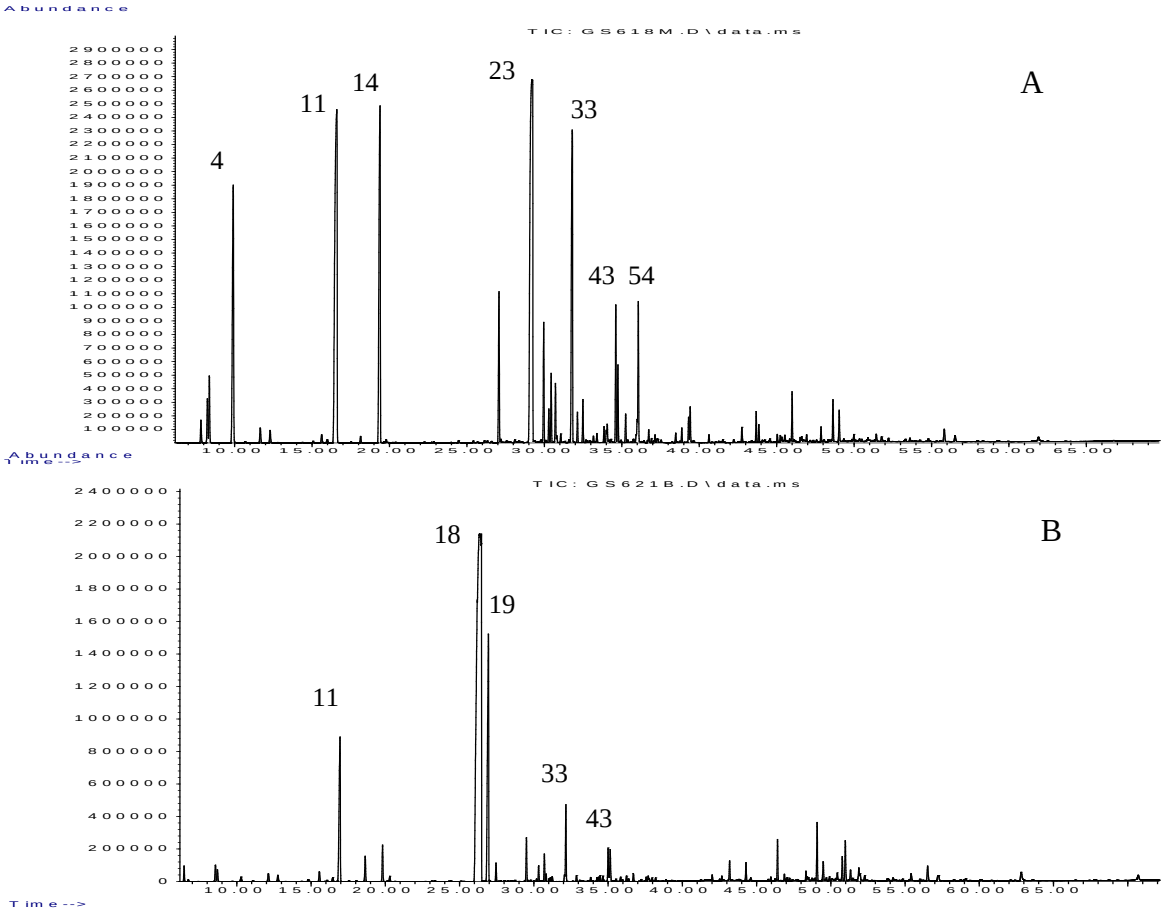

Figure 3. Chromatographic profile of the essential oils (A) and (B) of *Tanacetum praeteritm* ssp. *praeteritum*. Numeration of the peaks is depicted according to the list (Table 1) of detected compounds.

Results of the gas-chromatographic analyses of *T. praeteritum* ssp. *praeteritum* oils were compared with previously reported for this species actually and for *Tanacetum* species in general. Earlier, two subspecies, *T. praeteritum* ssp. *praeteritum* and *T. praeteritum* (Horwood) Heywood ssp. *massicyticum* Heywood collected in Muğla and Antalya provinces, respectively, have been investigated for the oils' chemical profiles. Noteworthy differences on the major compounds of the oils were detected. Namely, borneol (28.1%), 1,8-cineole (12.3%), bornyl acetate (10.0%), terpinen-4-ol (7.1%), β-pinene (5.7%) were reported for *T. praeteritum* ssp. *praeteritum.* The oil of *T. praeteritum* ssp. *massicyticum* was characterized with 1,8-cineole (4.0%), α- and β-thujone (51.1% and 10.0%, respectively) (19). It seems to be that the oils A and B investigated in the present work have different compositions than the previously reported ones. Borneol and bornyl acetate were detected in our samples in very scarce amounts. In our work, high abundance of camphor (>37%) was detected, while scarce amount of camphor was detected by Gönen et al. (19).

Although, oxygenated monoterpenes as major constituents have earlier been detected in different representatives of *Tanacetum* genus. The literature search revealed that α-thujone was found to be the major constituent in *T. argyrophyllum* var. *argyrophyllum* leaf (52%) and flower (63%) oils; in *T. argenteum* subsp. *canum* var. *canum* (12%) and *T. praeteritum* subsp. *massicyticum* (51%) oils (19). Table 2 summarizes *Tanacetum* species in which similar major constituents have earlier been detected. As can be seen from Table 2, the oxygenated monoterpenes such as camphor, 1,8-cineole, α- and β-thujone and borneol belong to common volatile constituents detected in *Tanacetum* oils.

Table 2. *Tanacetum* species with oxygenated monoterpenes as major volatile constituents (literature survey).

***Tanacetum* species**	**Main compounds (%)**	**Ref.**
T. argyrophyllum (C. Koch) Tvzel. var. *argyrophyllum* (C. Koch) Tvzel.	Camphor (26.6-29.7), 1,8-cineole (8.4-17.5), borneol (12.0-15.0)	(52)
	α-Thujone (52.0, 69.0)	(19, 53)
T. balsamita L. subsp. *balsamita* L.	β-Thujone (20.8)	(54)
T. chiliophyllum (Fisch. Et Mey.) Schultz Bip. var. *chiliophyllum (Fisch. Et Mey.)* Schultz	Camphor (19.7), 1,8-cineole (16.6), borneol (15.4)	(55)
	Camphor (17.0)	(56)
T. chiliophyllum (Fisch. et Mey.) Schultz Bip. var. *monocephalum* Grierson	1,8-Cineole (8.3), camphor (17.3)	(57)
T. densum (Lab.) Schultz Bip. subsp. *eginense* Heywood	Camphor (25.7-30.9)	(58)
T. densum (Lab.) Schultz Bip. subsp. *sivasicum* Hub.-Mor. et Grierson	1,8-Cineole (21.1, 28.3), camphor (19.2, 16.4), borneol (5.8, 6.4)	(59)
T. mucroniferum Hub. – Mor. et Grierson	1,8-Cineole (21.9), camphor (6.4)	(60)
T. parthenium (L.) Schultz Bip. varieties	Camphor (28-61)	(53, 61, 62)
T. tabrisianum (Boiss.) Sosn. et Takht	1,8-Cineole (17.6, 22.5)	(63)
T. vulgare L.	1,8-Cineole (10.8), camphor (30.5), borneol (14.8)	(9)

Biological activities of the oils

In the present work we evaluated the biological properties of *T. praeteritum* subsp. *praeteritum* essential oils, including antioxidant, antineurodegenerative, and antidiabetic effects *in vitro* using microplate titer assays. Antioxidant activity assessments were performed *in vitro* by using non-enzymatic systems employing different model substrates: Stable free radical $DPPH^{\bullet}$ and cation radical $ABTS^{+\bullet}$. Our results for antioxidant activities showed that the *T. praeteritum* subsp. *praeteritum* essential oils A and B had moderate (0.23 mM and 0.15 mM, respectively) Trolox equivalent antioxidant capacities. The oils demonstrated weak (3% and 8% inhibition) scavenging activities towards DPPH free radicals (Table 3).

Evaluation of *T. praeteritum* subsp. *praeteritum* oils for anti-neurodegenerative activity *via* inhibition of acetylcholinesterase revealed that the oil A showed higher inhibitory effect with an IC_{50} of 0.74 mg/mL than the oil B (IC_{50} of 1.78 mg/mL). Anti-cholinesterase effects

of 1,8-cineole, camphor and terpinen-4-ol which were the main constituents of the oil A have earlier been reported in a number of papers (34, 64-66).

A previous literature search revealed information about antidiabetic and enzyme inhibition properties of thujone (6, 67, 68). The use of thujone for the treatment of diabetes mellitus has recently been suggested by Baddar (6). Moreover, it was reported that the application of thujone appeared to have an effect similar to metformin; four-weeks treatment with thujone produced a pronounced hypoglycemic effect in alloxan diabetic rats (69). This information led us to the empirical search of the *T. praeteritum* subsp. *praeteritum* oils A and B to find additional therapeutic resources for diabetes treatment. The oils of *T. praeteritum* subsp. *praeteritum* were *in vitro* evaluated for hypoglycemic activity *via* inhibition of the porcine pancreatic α-amylase. So, previous testing showed that both the oils have noteworthy inhibitory activity (> 80%). Further serial dilution on microtiter plate allowed us to detect an IC_{50} values of the oils. As can be seen in Table 3, the oil B demonstrated higher inhibitory activity (IC_{50} of 0.89 ± 0.13 mg/mL) than the oil A (IC_{50} of 1.02 ± 0.24).

Table 3. Biological activities of *Tanacetum praeteritum* subsp. *praeteritum* essential oils.

Test sample	Antioxidant activity		Enzyme Inhibition	
	DPPH, Inh%	**TEAC, mM** ±SED	**AChE, IC_{50}**±SED	**α-Amylase, IC_{50}**±SED
Essential oil A	8	0.23±0.008	0.74±0.07	1.02±0.24
Essential oil B	3	0.152±0.035	1.78±0.16	0.89±0.13
Gallic acid	85	2.4	-	-
Galanthamine	-	-	0.01	-
Acarbose	-	-	-	0.08

CONCLUSION

Analysis of the chemical composition of *T. praeteritum* subsp. *praeteritum* essential oils demonstrated that they were mainly comprised of oxygenated monoterpenes such as camphor, borneol, 1,8-cineole, α- and β-thujone. In the present study, the essential oils were found to provide promising and effective alternative in the field of antineurodegenerative and antidiabetic applications. The oils demonstrated significant inhibitory potential on acetylcholinesterase and α-amylase enzymes, which involved into Alzheimer's disease and carbohydrate metabolism disorders. Finally, the essential oils inhibited ABTS cation radicals.

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REFERENCES

1.Grierson AJC. *Tanacetum* L. (emend. Briq.). In: Davis PH, editor. Flora of Turkey and The East Aegean Islands. 5. Edinburgh: Edinburgh University Press; 1975. p. 256-92

2.Bączek KB, Kosakowska O, Przybył JL, Pióro-Jabrucka E, Costa R, Mondello L, et al. Antibacterial and antioxidant activity of essential oils and extracts from costmary (*Tanacetum balsamita* L.) and tansy (*Tanacetum vulgare* L.). Ind. Crops Prod. 2017;102:154-63.

3.Godinho LS, Aleixo de Carvalho LS, Barbosa de Castro CC, Dias MM, Pinto PdF, Crotti AEM, et al. Anthelmintic activity of crude extract and essential oil of *Tanacetum vulgare* (Asteraceae) against adult worms of *Schistosoma mansoni*. Scientific World J. 2014;2014.

4.Haider SZ, Mohan M, Pandey AK, Singh P. Use of *Tanacetum tomentosum* and *Ta. dolichophyllum* essential oils as botanical repellents and insecticidal agents against storage pest *Tribolium castaneum* (Coleoptera: Tenebrionidae). Entomol. Res. 2017;47(5):318-27.

5.Polatoğlu K, Servi H, Yücel YY, Nalbantsoy A. Cytotoxicity and acetylcholinesterase inhibitory and PRAP activities of the essential oils of selected *Tanacetum* L. species. Nat. Volatiles Essent. Oils. 2015;2(4):11-6.

6.Baddar NWA-H, Aburjai TA, Taha MO, Disi AM. Thujone corrects cholesterol and triglyceride profiles in diabetic rat model. Nat. Prod. Res. 2011;25(12):1180-4.

7.de Almeida LMS, Carvalho LSAd, Gazolla MC, Silva Pinto PL, Silva MPNd, de Moraes J, et al. Flavonoids and sesquiterpene lactones from *Artemisia absinthium* and *Tanacetum parthenium* against *Schistosoma mansoni* worms. Evidence-Based Comp. Alterna. Medicine. 2016;2016:1-9.

8.Pålsson K, Jaenson TG, Bæckström P, Borg-Karlson A-K. Tick repellent substances in the essential oil of *Tanacetum vulgare*. J. Med. Entomol. 2008;45(1):88-93.

9.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):34.

10.Ozbilgin S, Akkol EK, Ergene Oz B, Ilhan M, Saltan G, Bahadir Acikara O, et al. 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. Ir. J. Basic Med. Sci. 2018;21(2):145-52.

11.Adekenov S. Sesquiterpene lactones with unusual structure. Their biogenesis and biological activity. Fitoterapia. 2017;121:16-30.

12.Carocho M, Ferreira IC. A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food Chem. Toxicol. 2013;51:15-25.

13.Pope C, Karanth S, Liu J. Pharmacology and toxicology of cholinesterase inhibitors: uses and misuses of a common mechanism of action. Environment. Tox. Pharmacol. 2005;19(3):433-46.

14.Pan H-J, Lin Y, Chen YE, Vance DE, Leiter EH. Adverse hepatic and cardiac responses to rosiglitazone in a new mouse model of type 2 diabetes: Relation to dysregulated phosphatidylcholine metabolism. Vasc. Pharmacol. 2006;45(1):65-71.

15.White JA, Hirsch IB. Nonhypoglycemic drug reactions of agents used to treat diabetes. Endocrin. Metabol. Clinics North Am. 2000;29(4):803-11.

16.Gören N. Flavonoids of *Tanacetum praeteritum* subsp. *praeteritum*. Journal of Faculty Pharmacy of Istanbul University. 1994;30:25-30.

17.Gören N. Sesquiterpene lactones from *Tanacetum praeteritum*. Phytochemistry. 1995;38(5):1261-4.

18.Gören N. Eudesmane-type sesquiterpenes from *Tanacetum praeteritum* subsp. *praeteritum*. Phytochemistry. 1996;42(3):747-9.

19.Gören N, Demirci B, Başer KHC. Composition of the essential oils of *Tanacetum* spp. from Turkey. Flavour and Fragrance Journal. 2001;16(3):191-4.

20.EDQM. Determination of Essential Oils in Herbal Drugs 9th ed. Europe Co, editor. Strasbourg: European Directorate for the Quality of Medicines and Healthcare; 2017. 285-6 p.

21.Özek G, Ishmuratova M, Tabanca N, Radwan MM, Göger F, Özek T, et al. One-step multiple component isolation from the oil of *Crinitaria tatarica* (Less.) Sojak by preparative capillary gas chromatography with characterization by spectroscopic and spectrometric techniques and evaluation of biological activity. J. Sep. Sci. 2012;35(5-6):650-60.

22.Joulain D, König WA, Hochmuth DH. Library of MassFinder-4. In: Hochmuth DH, editor. Terpenoids and Related Constituents of Essential oils. Hamburgh, Germany: Hochmuth Scientific Consulting; 2001.

23.Adams RP. Identification of Essential Oil Components by Gas Chromatography / Mass Spectrometry. 4th ed. Adams RP, editor: Allured Pub. Corp.; 2007. 804 p.

24.Baser KHC, Demirci F. Flavours and Fragrances.Chemistry of Essential Oils. In: Berger RG, editor. Flavours and Fragrances: Chemistry, Bioprocessing and Sustainability: Springer-Verlag Berlin Heidelberg; 2007. p. 648.

25.Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 1961;7(2):88IN191-9095.

26.Yang XW, Huang MZ, Jin YS, Sun LN, Song Y, Chen HS. Phenolics from *Bidens bipinnata* and their amylase inhibitory properties. Fitoterapia. 2012;83(7):1169-75.

27.Brand-Williams W, Cuvelier ME, Berset CLWT. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol. 1995;28(1):25-30.

28.Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Rad. Biol. Med. 1999;26(9):1231-7.

29.Orav A. Identification of terpenes by gas chromatography-mass spectrometry. In: Niessen WMA, editor. Current Practice of Gas-Chromatography Mass-Spectrometry Chromatographic Science Series. 86. Basel, Switzerland: Marcel Dekker AG; 2001. p. 483-94.

30.Maggio A, Rosselli S, Bruno M, Spadaro V, Raimondo FM, Senatore F. Chemical composition of essential oil from Italian populations of *Artemisia alba* Turra (Asteraceae). Molecules. 2012;17(9):10232-41.

31.Cha J-D, Jeong M-R, Jeong S-I, Moon S-E, Kim J-Y, Kil B-S, et al. Chemical composition and antimicrobial activity of the essential oils of *Artemisia scoparia* and *A. capillaris*. Planta Med. 2005;71(02):186-90.

32.Noorizadeh H, Farmany A, Noorizadeh M. Application of GA-PLS and GA-KPLS calculations for the prediction of the retention indices of essential oils. Química Nova. 2011;34(8):1398-404.

33.Bignell C, Dunlop P, Brophy J. Volatile leaf oils of some south-western and southern Australian species of the genus *Eucalyptus* (series 1). Part XIX. Flavour Fragr. J. 1998;13(2):131-9.

34.López MD, Campoy FJ, Pascual-Villalobos MJ, Muñoz-Delgado E, Vidal CJ. Acetylcholinesterase activity of electric eel is increased or decreased by selected monoterpenoids and phenylpropanoids in a concentration-dependent manner. Chem. Biol. Interact. 2015;229:36-43.

35.Bader A, Panizzi L, Cioni PL, Flamini G. *Achillea ligustica:* composition and antimicrobial activity of essential oils from the leaves, flowers and some pure constituents. Central Eur. J. Biol. 2007;2(2):206-12.

36.Chéraif I, Jannet HB, Hammami M, Khouja M, Mighri Z. Chemical composition and antimicrobial activity of essential oils of *Cupressus arizonica* Greene. Biochem. Syst. Ecol. 2007;35(12):813-20.

37.Noorizadeh H, Farmany A. Exploration of linear and nonlinear modeling techniques to predict of retention index of essential oils. J. Chinese Chem. Soc. 2010;57(6):1268-77.

38.Tan N, Yazici-Tutunis S, Yesil Y, Demirci B, Tan E. Antibacterial activities and composition of the essential oils of *Salvia sericeo-tomentosa* varieties. Rec. Nat. Prod. 2017;11:456-61.

39.Baser K, Demirci B, Ozek T, Viljoen A, Victor J. Composition of the essential oils of five *Coleonema* species from South Africa. J. Essent. Oil Res. 2006;18:26-9.

40.Demirci F, Demirci B, Gürbüz I, Yesilada E, Baser KHC. Characterization and biological activity of *Achillea teretifolia* Willd. and *A. nobilis* L. subsp. *neilreichii* (Kerner) Formanek essential oils. Turk. J. Biol. 2009;33(2):129-36.

41.Arze JBL, Collin G, Garneau F-X, Jean F-I, Gagnon H. Essential Oils from Bolivia. VI. Lamiaceae: *Lepechinia graveolens* (Reg.) Epling *L. floribunda* (Benth.) Epling, and *L. meyeni* (Walp.) Epling. J. Essent. Oil Res. 2009;21(1):36-40.

42.Nam AM, Tomi F, Gibernau M, Casanova J, Bighelli A. Composition and chemical variability of the needle oil from *Pinus halepensis* growing in Corsica. Chem. Biodivers. 2016;13(4):380-6.

43.Lopes-Lutz D, Alviano DS, Alviano CS, Kolodziejczyk PP. Screening of chemical composition, antimicrobial and antioxidant activities of Artemisia essential oils. Phytochemistry. 2008;69(8):1732-8.

44.Collin G. Aromas from Quebec. IV. Chemical composition of the essential oil of *Ledum groenlandicum*: A review. Am. J. Essent. Oils Nat. Prod. 2015;2(3):06-11.

45.Fang Y, Qian M. Aroma compounds in Oregon Pinot Noir wine determined by aroma extract dilution analysis (AEDA). Flavour Fragr. J. 2005;20(1):22-9.

46.Kurose K, Okamura D, Yatagai M. Composition of the essential oils from the leaves of nine Pinus species and the cones of three of Pinus species. Flavour Fragr. J. 2007;22(1):10-20.

47.Tabanca N, Avonto C, Wang M, Parcher JF, Ali A, Demirci B, et al. Comparative investigation of *Umbellularia californica* and *Laurus nobilis* leaf essential oils and identification of constituents active against *Aedes aegypti*. J. Agric. Food Chem. 2013;61(50):12283-91.

48.Perry PL, Wang Y, Lin J. Analysis of honeydew melon (Cucumis melo var. inodorus) flavour and GC–MS/MS identification of (E, Z)-2, 6-nonadienyl acetate. Flavour Fragr. J. 2009;24(6):341-7.

49.Baser KHC, Demirci B, Iscan G, Hashimoto T, Demirci F, Noma Y, et al. The essential oil constituents and antimicrobial activity of *Anthemis aciphylla* Boiss. var. *discoidea* Boiss. Chem. Pharm. Bull. 2006;54(2):222-5.

50.Öztürk B, Özek G, Özek T, Baser KHC. Chemical diversity in volatiles of *Helichrysum plicatum* DC. subspecies in Turkey. Rec. Nat. Prod. 2014;8(4):373.

51.Bousbia N, Vian MA, Ferhat MA, Petitcolas E, Meklati BY, Chemat F. Comparison of two isolation methods for essential oil from rosemary leaves: Hydrodistillation and microwave hydrodiffusion and gravity. Food Chem. 2009;114(1):355-62.

52.Polatoglu K, Demirci F, Demirci B, Gören N, Baser KHC. Antimicrobial activity and essential oil composition of a new *T. argyrophyllum* (C. Koch) Tvzel var. a*rgyrophyllu*m chemotype. J. Oleo Sci. 2010;59(6):307-13.

53.Akpulat HA, Tepe B, Sokmen A, Daferera D, Polissiou M. Composition of the essential oils of *Tanacetum argyrophyllum* (C. Koch) Tvzel. var. *argyrophyllum* and *Tanacetum parthenium* (L.) Schultz Bip.(Asteraceae) from Turkey. Biochem. Syst. Ecol. 2005;33(5):511-6.

54.Yousefzadi M, Ebrahimi SN, Sonboli A, Miraghasi F, Ghiasi S, Arman M, et al. Cytotoxicity, antimicrobial activity and composition of essential oil from *Tanacetum balsamita* L. subsp. *balsamita*. Nat. Prod. Comm. 2009;4(1):119-22.

55.Salamci E, Kordali S, Kotan R, Cakir A, Kaya Y. Chemical compositions, antimicrobial and herbicidal effects of essential oils isolated from Turkish *Tanacetum aucheranum* and *Tanacetum chiliophyllum* var. *chiliophyllum*. Biochem. Syst. Ecol. 2007;35(9):569-81.

56.Başer K, Demirci B, Tabanca N, Özek T, Gören N. Composition of the essential oils of *Tanacetum armenum* (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 Fragr. J. 2001;16(3):195-200.

57.Polatoğlu K, Demirci B, Demirci F, Gören N, Başer KHC. Biological activity and essential oil composition of two new *Tanacetum chiliophyllum* (Fisch. & Mey.) Schultz Bip. var. *chiliophyllum* chemotypes from Turkey. Ind. Crops Prod. 2012;39:97-105.

58.Polatoglu K, Demirci B, Demirci F, Gören N, Baser KHC. The essential oil composition of *Tanacetum densum* (Labill.) Heywood ssp. *eginense* Heywood from Turkey. Rec. Nat. Prod. 2012;6(4):402.

59.Polatoğlu K, Gören N, Başer KHC, Demirci B. The essential oil composition of *Tanacetum densum* (Labill.) Heywood ssp. *sivasicum* Hub.-Mor. & Grierson from Turkey. J. Essent. Oil Res. 2009;21(3):200-2.

60.Polatoglu K, Sen A, Kandemir A, Gören N. Essential oil composition and DPPH scavenging activity of endemic *Tanacetum mucroniferum* Hub.–Mor. & Grierson from Turkey. J. Essent. Oil Bearing Plants. 2012;15(1):66-74.

61.Hendriks H, Bos R, Woerdenbag H. The Essential Oil of *Tanacetum parthenium* (L.) Schultz-Bip. Flavour Fragr. J. 1996;11(6):367-71.

62.Polatoglu K, Demirci F, Demirci B, Gören N, Baser KHC. Antibacterial activity and the variation of *Tanacetum parthenium* (L.) Schultz Bip. essential oils from Turkey. J. Oleo Sci. 2010;59(4):177-84.

63.Polatoğlu K, Demirci B, Gören N, Başer KHC. Essential oil composition of endemic *Tanacetum zahlbruckneri* (Náb.) and *Tanacetum tabrisianum* (Boiss.) Sosn. and Takht. from Turkey. Nat. Prod. Res. 2011;25(6):576-84.

64.Dohi S, Terasaki M, Makino M. Acetylcholinesterase inhibitory activity and chemical composition of commercial essential oils. J Agric Food Chem. 2009;57(10):4313-8.

65.Kaufmann D, Dogra AK, Wink M. Myrtenal inhibits acetylcholinesterase, a known Alzheimer target. J Pharm Pharmacol. 2011;63(10):1368-71.

66.Awasthi M, Upadhyay AK, Singh S, Pandey VP, Dwivedi UN. Terpenoids as promising therapeutic molecules against Alzheimer's disease: amyloid beta-and acetylcholinesterase-directed pharmacokinetic and molecular docking analyses. Molecular Simulation. 2017:1-11.

67.Daradka HM, Abas MM, Mohammad MA, Jaffar MM. Antidiabetic effect of *Artemisia absinthium* extracts on alloxan-induced diabetic rats. Comparative Clinical Pathology. 2014;23(6):1733-42.

68.Lachenmeier DW, Walch SG. The choice of thujone as drug for diabetes: Reply to: Al-Haj Baddar et al.(2011). Natural Product Research. 2011;25(20):1890-2.

69.Al-Omari SM. The effect of thujone and myrcene on diabetes mellitus in albino rats. Faculty of Graduate Studies University of Jordan. 2007; Master's Degree of Science in Biological Sciences.