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Research Article

Evaluation of the Phytochemical and Antioxidant Potential of Aerial Parts of Iranian *Tanacetum parthenium*

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ABSTRACT

Background: The objective of this study was to analyze the essential oil, fatty acid, flavonoid, phenolic compounds and in vitro antioxidant activity of oil from Feverfew (*Tanacetum parthenium* L.) wild grown and collected from north of Iran.
Methods: The essential oil of aerial parts was analyzed by spectroscopy method (GC/MS using HP-5MS column) while the fatty acid content was analyzed by gas chromatography (GC/FID). Phenolic contents of the oil were evaluated using high performance liquid chromatography (HPLC/UV) technique while total phenols and flavonoids were determined colorimetrically. The in vitro antioxidant activity of the essential oil was evaluated by 1,1-diphenyl-2 picryl hydrazyl (DPPH) radical scavenging technique.
Results: In the essential oil thirteen compounds were characterized with camphor (43.97 %), chrysanthenyl acetate (12.46 %) and farnesol (7.54%) as the major components. Principal fatty acid components of the herb were palmitic acid (57.27%) and myristic acid (14.7%). HPLC analysis revealed that the cinnamic acid derivatives were the major compounds, with sinapic (3.86 ± 0.1 mg/g dw) and ferulic (2.59 ± 0.1 mg/g dw) acids being the predominant ones. Also, evaluation the bioactivity of the oil showed considerable antioxidant capacity (TPC = 152.8 ± 0.8 mg/g and DPPH = 73.8 ± 1.3 %).
Conclusion: This study revealed that the essential oil was rich in camphor/chrysanthenyl acetate chemotype and different polyphenols in the category of hydroxycinnamic acid derivatives. In addition, this research demonstrated that the aerial parts of this aromatic herb were various sources of oily components, especially essential fatty acids.

Introduction

The medicinal plants are useful for curing of various human diseases as well as for healing because of the presence of phytochemical constituents.[1] Phytochemicals are classified based on their chemical class, functional groups and biosynthetic origin into primary and secondary metabolites.[2] Primary metabolites (like amino acids, fatty acids etc) have a key role in metabolic processes such as respiration, photosynthesis and nutrient assimilation which are used as industrial raw materials and food additives.[2,3] Secondary metabolites are not directly involved in the normal life cycle but help the plant adjust to the surroundings. The most important secondary metabolites are terpenoid, alkaloids and phenolic compounds which are considered as valuable basic constituents for food and pharmaceutical industries.[2,4] On one hand, in addition to genetic differences, environmental conditions such as developmental stage, sun exposure, temperature, nutritional variation, water supply, and the presence of microorganisms, affect the cellular processes in the medicinal herbs and their responses to stimuli.[3,5] on the other hand, plant-based foods are complex mixtures of bioactive compounds, information on the potential health effects of individual phytochemicals is linked to information on the health effects of foods that contain those phytochemicals.[3,6,7] Hence, phytochemical analysis of the plants is very important for nutritional and pharmaceutical applications.[1,8] Feverfew (*Tanacetum parthenium* L.) is a perennial herbaceous plant belonging to the aster family, Asteraceae, and has a wide distribution range in Asia, Europe and America.[9] It

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is distributed in various regions of North, West, East and center of Iran[10] which have several therapeutic properties like as anti-septic, anti-microbial, anti-parasitic and anti-inflammatory properties.[11,12] Also, it had been used us an insect repellant and had antioxidant, antifungal and antibacterial activity.[12,13] Flavonoids, parthenolide and a number of related sesquiterpene lactones considered to be responsible for these activities.[12] There are several reports on the volatile oil composition of *T. parthenium*. In most cases, camphor (42-60%), chrysanthenyl acetate (13-25%), camphen (1.5-12%) and *p*-Cymene (0.1-5.2%) are the main components together with various secondary components.[11,12,14,15] Other scientific data (such as phenolic and fatty acid compounds) on the genus are very limited. Therefore, The objective of this study was to analyze the essential oil, fatty acids, flavonoid, phenolic compounds and antioxidant activity of *T. parthenium* oil grown collected from Guilan, North of Iran.

Materials and Methods

Collection of plant material

Samples of the aerial parts of *Tanacetum parthenium* were manually collected in June 2016 in Masuleh hills (970 m above sea level and N: 37° 9′ 15″ , E:48° 59′ 24″) in Guilan province, North of Iran. A voucher specimen (identification no: 5544) was deposited in the Herbarium of the Guilan Agricultural Research Center (GARC) and identified by Dr. Morady (taxonomist). Before oil isolation, the plant materials were dried, in a forced-air drier (20-25°C), for one week until constant weight.

GC-MS analysis conditions

The collected aerial parts (100g) were hydrodistilled for 3 h using a Clevenger-type system. Samples were dried with anhydrous sodium sulfate and kept in closed sterilized glass vials at 4ºC until chromatographic analysis (GC/MS method). Gas chromatography-mass spectrometry (GC-MS) analysis was carried out on an Agilent Technologies 5973 gas chromatograph fitted with a HP-5MS 5% capillary column (30 m×0.25 mm, 0.25 µm film thicknesses). Carrier gas was helium at flow of 0.8 mL/min. GC oven temperature was kept at 120°C for 5 min and then programmed to 260 °C at a rate of 15 °C/min. The injector temperature was set at 250 °C. The purity of Helium gas was 99.99% and 1 µL samples were injected manually in the split mode. Mass spectra were recorded at 70 eV and scanned in the range of 30-300 amu. Identification of oil components was accomplished based on comparison of their retention times (RT) with those of authentic standards and by comparison of their mass spectral fragmentation patterns (Wiley7n.1 and NIST 2008).[16]

GC analysis conditions

Fatty acid methyl esters (FAMEs) were prepared using 2 mol/L NaOH in methanol and n-heptane. Samples of 1µL were subjected to analysis by capillary gas chromatography. A Beifen SP-3420A, gas chromatograph equipped with a flame ionization detector (FID) and a 30 m x 0.25 mm BP (cross-linked polyethylene glycol) column with 0.25 µm film thickness, was used for this study. The FID and the injector were maintained at 280ºC and 250ºC, respectively. Nitrogen was used as carrier gas, the flow through the column was 1.8 mL/min, and the split ratio was set to 1:10. Oven temperature 100-230 ºC with the rate of 10 ºC /min. For the identification of the compounds, retention times and retention index were confirmed with commercially available standard compounds (Sigma, Chemical Co.St. Louis).

HPLC analysis conditions

A 20 µL aliquot of sample solution (the methanolic solution of the essential oil) was separated using a HPLC system (Knauer-Germany) equipped with UV-Vis multiwavelength detector and a eurospher 100-5 C-18 column (25 cm × 4.6 mm; 5 µm). The mobile phase consisted of purified water with 2% acetic acid (solvent A) and acetonitrile (solvent B) at a flow rate of 0.8 mL/min. The solvent gradient elution program was as follows: 0.8 mL/min flow rate and the temperature was set at 25 °C, isocratic conditions from 0 to 5 min with 85 % A flow, from 5 to 15 min a linear gradient of 85 % A to 100 % B. After termination of the cycle, 15 min of column equilibration (85 % A) were allowed prior next injection. The detection and quantification phenolic compounds was done at 280 nm.[17] Concentration of each individual compound was calculated using an external standard method and was converted to mg compound per g dry weight (dw).

Total phenolic assay

The amount of total phenolic (TPC) of the essential oil was determined using Folin-Ciocalteu reagent, as described by Kahkonen et al.[18] Briefly, the samples (20 µL) were made up to 1 mL of the Folin-Ciocalteu reagent were neutralized with 0.8 mL of sodium carbonate solution (7.5%, w/v). The mixture was allowed to stand for a further 30 min in the dark, and absorbance was measured at 765 nm (WPA Biowave S2100). The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of gallic acid equivalent per g dry weight (The calibration equation for Gallic acid:

$y = 0.0421 x - 0.0232$, $R^2 = 0.998$ Eq.(1).

Table 1. Main components of the essential oil from *T. parthenium.*

Compounds	RI*	Formula	Percent
p-Cymene	1027	$C_{10}H_{14}$	3.6
Limonene	1032	$C_{10}H_{16}$	2.5
Camphor	1145	$C_{10}H_{16}O$	43.97
Borneol	1167	$C_{10}H_{18}O$	2.16
trans-Carveol	1218	$C_{10}H_{16}O$	1.23
Chrysanthenyl Acetate	1240	$C_{12}H_{18}O_2$	12.46
Germacrene D	1483	$C_{15}H_{24}$	0.96
Valencene	1491	$C_{15}H_{24}$	6.67
Germacrene B	1554	$C_{15}H_{24}$	6.44
Cycloisolongifolene8-hydroxy-endo	1608	$C_{15}H_{24}O$	6.73
Caryophyllenol II	1675	$C_{15}H_{24}O$	2.34
Farnesol	1712	$C_{15}H_{26}O$	7.54
2(1H)Naphthalenonhexahydro-4,8a-dimethyl-6-(1-methylethenyl)	1773	$C_{15}H_{22}O$	2.47
Oxygenated monoterpenes			59.82
Monoterpene hydrocarbons			5.75
Oxygenated sesquiterpenes			19.08
Sesquiterpene hydrocarbons			14.07
Total			98.72

RI* = Retention indices as determined on HP-5MS column.

Total flavonoid content

The total flavonoid content (TFC) of the essential oil was determined by the aluminium chloride colorimetric method.[19] In brief, the samples (20 µL) were added to 0.3 mL distilled water followed by 5% NaNO2 solution (75 µL). After 5 min at 25 ºC, 10% AlCl3 (0.15 mL,) solution was added. After further 5 min, the reaction mixture was treated with 0.5 mL of 1 mol/L NaOH. The final volume of the mixture was brought to 3 mL with deionized water and absorbance was measured at 510 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg rutin equivalent per g dry weight (The calibration equation for quercetin:

$y = 0.0779 x - 0.0136, R^2 = 0.998$ Eq.(2)

DPPH radical scavenging assay

The antioxidant activity of the oil was determined by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay, as described earlier by Hatano et al.[20] Briefly,10 µL of the EOs were mixed with 2 mL DPPH (0.0023 mol/L) and incubated in the dark at room temperature for 30 min. The absorbance of the mixture was then measured at 520 nm. Pure methanol was used to calibrate the spectrophotometer and ascorbic acid was used as a positive control. The ability of the sample to scavenge DPPH radical was determined from:

$\%\ \text{inhibition} = [(AC - AS)/AC] \times 100$ Eq.(3)

where AC is the absorbance of control reaction and AS is the absorbance of the sample at 520 nm.

Data analysis

All the samples were done in triplicate, except those for GC-MS and GC methods which were analyzed once. All data were recorded as means ± SE and analysed by SPSS for Windows (version 17). One-way analysis of variance (ANOVA, $P < 0.05$) and Duncan comparisons were carried out to test any significant differences between samples.

Results and Discussion

Essential oil composition

This is the first report of oil composition of wild *T. parthenium* in Northern Iran. Detailed results pertaining to components and their contents (%) of different oils are presented in Table 1. The amount of the oil obtained was 3.5% (based on one hundred grams of dry weight) in the flowering stage. The percentage yield of the oil herb was slightly different from the previously reported data.[15,21] Results showed that essential oil of the feverfew had 13 compounds which had formed 98.72 percent of the entire essential oil. Camphor (43.97%), chrysanthenyl acetate (12.46%) and farnesol (7.54%) were the main components of the essential oil among identified compounds. As can be seen from the above table, the total amounts of monoterpene fractions in the oil (65.57%) were higher than sesqueiterpenes fractions (33.15%). Differences can be clearly seen on the major constituents of *T. parthenium* volatile oil from the previous literature,[22,23] and our work in Table 1.

For example, 1,8-cineole is the main component in Germany and Italy with the values of 59.9% and 37.3% respectively; Also, α-pinene and camphor with the values of 19.6% and 14% respectively in Germany and 10.3% and 9.9% in Italy were characterized as the second and third major

compounds; and farnesol and chrysanthenyl acetate were not found in the essential oil.

Also, according to the reports on *T. parthenium* growing in Iran showed similarities in terms of composition of the major constituents of this herb and differences in terms of the percentage of these compositions.[14,15,21,24]

In comparison with one of the analyses of the oil of roots from *T. parthenium* (collected from Karaj), camphor and chrysanthenyl acetate were identifid as 30.2% and 26.5%, respectively. Monoterpenoids were the main components of the oil (66.5%), but sesquiterpenoides (20%) had low percent.[14] In another investigation from Ardabil province on oil of flowers of *T. parthenium*, camphor (61.1%) and camphene (9.2 %) were the major constituents. In addition, the oil obtained of the plant was found oxygenated monoterpenes (75.7 %) and oxygenated sesquiterpenes (5.6 %).[21] Essential oils of aerial parts of the herb from Tehran province were investigated by Mirjalili et al.[24] The major constituents were camphor (50.5%) and germacrene-D (9.2%). As, oxygenated monoterpenes were the main group of compounds in the oil (85.9%). The comparison of the present results with earlier reports showed similarity in the presence of the main component. [14,21,24,25] It seems that camphor is the major constituent of Iranian *T. parthenium* oil. however, the volatile oil showed considerable difference in other constituents, especially due to farnesol (7.54%), cycloisolongifolene8-hydroxy-endo (6.73%), valencene (6.67%), germacrene B (6.44%), etc., which some of them are not reported in earlier studies on *T. parthenium* oil from Iran. Also, results of present study revealed that, increasing the amount of a sesquitrepene can lead to a monoterpene amount decreases. However, the above mentioned studies display that these differences in the quantity or quality of the oil composition could be related to ecological factors, the vegetative period, plant tissue, climatic and soil conditions, different collection times, methods and instruments employed in analysis. In conclusion, this study demonstrates the occurrence of camphor/chrysanthenyl acetate chemotype of *T. parthenium* in Northern region of Iran.

Fatty acids profiles

Vegetable oils are rich source of fatty acids and they have found wide applications in many branches of industry, in particular in food industry and cosmetic, pharmacy and medicine.[26] The fatty acid composition of the aerial parts oil are presented in Table 2 that analyzed using a GC-FID for the first time from Iran. n-heptane extraction of *T. parthenium* herb aerial parts yielded 36% oil. The dominant fatty acids were palmitic acid (57.27 %), and myristic acid (14.7 %) in the oil. Other fatty acids with low concentrations were lauric (7.37%), capric (5.39%), arachidic (1.74%), stearic (1.32%), lignoceric (0.2%), and unsaturated fatty (12.04 %) derivatives respectively. Tsevegsuren et al.[27] reported (GLC separation method) that a new conjugated *trans,trans*-diunsaturated acetylenic acid (17%), found for the first time in nature as a main component of the seed oil of *Tanacetum corymbosum*, was shown to be octadeca-8*t*,10*t*-dien-12-ynoic acid. Also, they were showed that the oil contain crepenynic acid (10%), palmitic acid (4.2 %), stearic acid (1.6%) and arachidic (0.3%). Comparing between the fatty acid profiles obtained from current study with fatty acids previous reported on this genus some differences were observed. It seems that these valuable metabolites are affected by both genetic and environmental factors.[26] However, vegetable oils play important functional and sensory roles in food products, and they also provide energy and the essential fatty acids (monounsaturated or polyunsaturated), responsible for growth.[26]

Table 2. Fatty acids profiles in *T. parthenium* oil.

No	Fatty acid	Acronym	Concentration (%)
1	Capric acid	C10:0	5.39
2	Lauric acid	C12:0	7.34
3	Myristic acid	C14:0	14.7
4	Palmitic acid	C16:0	57.27
5	Palmitoleic acid	C16:1	1.79
6	Stearic acid	C18:0	1.32
7	Oleic acid	C18:1	0.01
8	Linoleic acid	C18:2	6.03
9	Linolenic acid	C18:3	2.01
10	Arachidic acid	C20:0	1.74
11	Behenic acid	C22:0	0.00
12	Erucic acid	C22:1	2.2
13	Lignoceric acid	C24:0	0.2
TSFA		87.96	
TUFA		12.04	
TUFA/TSFA		0.14	

TSFA: Total saturated fatty acids
TUFA: Total unsaturated fatty acids

HPLC-UV quantitative analysis

The HPLC analysis revealed the presence of various compounds in the essential oil of wild *T. parthenium* for the first time from Iran and abroad (Table 3). The HPLC chromatograms recorded at 280 nm confirmed the presence of 4-hydroxy benzoic acid (3.92±0.3 mg/g), sinapic acid (3.86± 0.2 mg/g), ferulic acid (2.59±0.1 mg/g), *p*-coumaric acid (2.05±0.2 mg/g), syringic acid (1.96±0.1 mg/g) and vanillic acid (0.18±0.1 mg/g) as the main compounds in the plant. In addition, in studied plant rutin, caffeic acid and gallic acid were not separated following the same method. In a study in Iran on ethanolic extract of six species of *Tanacetum* (*T. budjnurdense*, *T. hololeucum*, *T.*

chiliophyllum, *T. sonboli*, *T. tabrisianum*, *T. kotschyi*), caffeic acid, ferulic acid, luteolin, apigenin and rutin were detected as major phenolic compounds in all the species investigated.[28] The phenolic acids found in this study are known to have many important biological and pharmacological properties and may have benefits for human health.[29]

Phenolic (TPC) and flavonoid (TFC) contents

The key role of phenolic and flavonoid components as antioxidants is emphasized in several reports.[2,4,12] Therefore, it would be valuable to determine these antioxidants of the plant oil. To our knowledge, there are no publisheds report on total Phenolic and flavonoid contents of wild *T. parthenium* essential oil in Iran. As shown in Table 4, total phenolic contents in the oil of *A. millefolium* calculated from the calibration curve (R^2 =0.9988), was 152.2 ± 0.8 mg GAE/g dry plant sample ($p < 0.05$). Also The content of flavonoid compounds (R^2 = 0.9978) in the herb was found to be 70.2 ± 0.3 mg QE/g ($p < 0.05$). There are no reports in assessment these factors in the oil, but about other species of *Tanacetum*, Malekpoor et al.[30] reported that amount of total phenolic of essential oil of *Tanacenatum polycephalum* varied from 0.063 to 0.153 (mg galic acid g dw). In addition, Wu et al.[31] reported that the amount total phenolic content of the alcoholic extract of *T. parthenium* was measured in 21.21 µg GAE/mg dw. Also, in a study in Iran on *T. sonbolii* species different extracts was found that TFC varied from 1.5 to 41.3 µg Q Es/mg.[31] In the assays, content of flavonoids of the test samples followed the order hexane (41.3±0.2) > methanol (37.1±0.8) > ethyl acetate (26.5±0.1) > chloroform (5.1±0.1) > butanol (1.9±0.0) > water (1.5±0.1). Thus, the comparative study showed that the essential oil the herb can be considered as useful sources of natural antioxidant for pharmaceutical industries and as antioxidant food preservatives. However, quantityplant and composition of the polyphenols such as flavonoid and phenolics vary significantly according to different extrinsic and intrinsic factors, such as soil and growing conditions, plant genetics, harvesting time and the plant part used.[4,12,30,32]

DPPH radical scavenging assay

The DPPH test is widely used in assessing antioxidants because of the ease of the reaction.[11,32] In our study, the antioxidant activity of essential oil of *T. parthenium* was expressed as IC_{50} with value 30.23 ± 0.8 µg/mL (73.8 ± 1.3%) that indicating the essential oil acts as considerable DPPH scavenger (Table 4). According to the report on *T. parthenium* from two different localities in Turkey,[11] when compared to the positive control Savsat oil (17.3%) showed low and Davutpasa oil (59.3%) showed medium DPPH scavenging activity. However, our results showed that antioxidant activity of the oil was 73.8 ± 1.3% more than reported by Polatoglu et al.[11] The results suggest that the high scavenging activities of the oils are due to the content of monoterpene and sesquiterpene alcohols.[33] The results of this study indicated that the essential oil of *T. parthenium* can be suggested as a natural antioxidant for the nutritional and pharmaceutical industries.

Table 3. Content of phenolic compounds in essential oil of *T. parthenium.*

Phenolic compound	Calibration curve*	R^2	Sample (mg/g DW)
Rutin	Y=2011928x-1392956	0.998	-
Gallic acid	Y=2372008.6x-1576952.1	0.997	-
Caffeic acid	Y=1278406.2x-1853153.7	0.988	-
4-Hydroxy benzoic acid	Y=762895x-733317	0.998	3.92 ± 0.3
Vanillic acid	Y=3159050.4x-296093	0.999	0.18 ± 0.1
P-coumaric acid	Y=82887x-59041	0.998	2.05 ± 0.2
Syringic acid	Y=13571x-3682.9	0.985	1.96 ± 0.1
Ferulic acid	Y=165138x-136553	0.988	2.59 ± 0.1
Sinapic acid	Y=20727x-9590	0.977	3.86 ± 0.2

*Linear calibration curve for HPLC-UV analysis of the phenolic compounds. Each value is the mean ± SD (n=3).

Table 4. The content of total polyphenols, flavonoids and antioxidant capacity parameters in the plant.

Samples	TPC(mg GAE/g)	TFC(mg QUE/g)	DPPH(%)	IC_{50}(µg/ml)
Essential oil	152.2±0.8	70.2±0.3	73.8±1.3^a	30.23±0.8^b
Gallic acid	-	-	93.12±0.4^b	0.15±0.01^a
Ascorbic acid	-	-	92.30±0.2^b	0.16±0.01^a

Each value is the mean ± SD of three independent measurements. Values in the same column followed by a different letter (a,b) are significantly different (p<0.05). GAE: Gallic acid equivalents; QUE: Quercetin equivalents.

However, volatile oils are complex mixtures of many components (such as alcohols, terpenes, phenols, epoxides, acids, esters and phenylpropanoids) that can fluctuate in quantity, quality, and composition according to soil composition, climate, plant organ , harvesting time, age, and growth stage plant.[11,33,34]

Conclusion

The results of this study demonstrated the occurrence of camphor/chrysanthenyl acetate chemotype of *T. parthenium* in Northern region of Iran. Also, HPLC analysis revealed that different polyphenols in the category of hydroxycinnamic acid derivatives were the major compounds in the oil. Therefore, the data obtained suggested that the essential oil of the plant could be used as easily accessible source of natural antioxidants, but also as a possible food supplement or in pharmaceutical industry. In addition, this research work revealed that the aerial parts of the species are various sources of oily components, especially the essential ones that are important for the nutrition sciences, because fatty acids seem to have considerable effect on health.

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Conflict of interests

The authors claim that there is no conflict of interest.

References

1. Nostro A, Germano MP, Dangelo V, Marino A, Cannatelli MA. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett Appl Microbiol. 2000;30(5):379-84. doi:10.1046/j.1472-765x.2000.00731.x
2. Krishnaiah D, Sarbatly R, Bono A. Phytochemical antioxidants for health and medicine: A move towards nature. Biotechnol Mol Biol Rev. 2007;1(4):97-104.
3. Lee DK, Yoon MH, Kang YP, Yu J, Park JH, Lee J, et al. Comparison of primary and secondary metabolites for suitability to discriminate the origins of *Schisandra chinensis* by GC/MS and LC/MS. Food Chem. 2013; 141(4):3931-7. doi:10.1016/j.foodchem.2013.06.064
4. RoepenackLahaye EV, Degenkolb T, Zerjeski M, Franz M, Roth U, Wessjohann L, et al. Profiling of Arabidopsis secondarymetabolites by capillary liquid chromatography coupled to electrospray ionization quadrupole time-of-flight massspectrometry. Plant Physiol. 2004;134(2):548-59. doi:10.1104/pp.103.032714
5. Kokale CK, Purohit AP, Gokhale SB. Practical Pharmacognosy. 2th ed. Vallabh Prakashan: New Delhi; 2004. p. 466-70.
6. Mihalik SJ, Michaliszyn SF, de las Heras J, Bacha F, Lee S, Chace DH, et al. Metabolomic profiling of fatty acid and amino acid metabolism in youth with obesity and type 2 diabetes. Diabetes Care. 2012;35(3):605-11. doi:10.2337/dc11-1577
7. Sun H, Zhang A,Wang X. Potential role of metabolomic approaches for Chinese medicine syndromes and herbal medicine. Phytother Res. 2012;26(10):1466-71. doi:10.1002/ptr.4613
8. Gika HG, Theodoridis GA, Wingate JE, Wilson ID. Within-day reproducibility of an HPLC–MS-based method for metabolomic analysis: Application to human urine. J Proteome Res. 2007;6(8):3291-303. doi:10.1021/pr070183p
9. Paulsen E, Christensen LP, Andersen KE. Do monoterpenes released from feverfew (Tanacetum parthenium) plants cause airborne Compositae dermatitis?. Contact Dermatitis. 2002;47(1):14-8. doi:10.1034/j.1600-0536.2002.470103.x
10. Mozaffarian V. Dictionary of Iranian Plant Names. Tehran: Farhang Moaser Publishers; 2007.
11. Polatoglu K, Demirci F, Demirci B, Gören1 N, Baser K. Antibacterial activity and the variation of *Tanacetum parthenium* (L.) Schultz Bip. Essential oils from Turkey. J Oleo Sci. 2010;59(4):177-84. doi:10.5650/jos.59.177
12. Pareek A, Suthar M, Rathore GS, Bansal V. Feverfew (*Tanacetum parthenium* L.): A systematic review. Pharmacogn Rev. 2011;5(9):103-10. doi:10.4103/0973-7847.79105
13. Bandoniene D, Pukalskas A, Venskutonis PR, Gruzdiene D. Preliminary screening of antioxidant activity of some plant extracts in rapeseed oil. Food Res Int. 2000;33(9):785-91. doi:10.1016/s0963-9969(00)00084-3
14. Mojab F, Tabatabai SA, Naghdi-Badi H, Nickavar B, Ghadyani F. Essential oil of the root of *Tanacetum parthenium* (L.) Schulz. Bip. (Asteraceae) from Iran. Iran J Pharm Res. 2007;6(4):291-3.
15. Mohsenzadeh F, Chehregani A, Amiri H. Chemical composition, antibacterial activity and cytotoxicity of essential oils of *Tanacetum parthenium* in different developmental stages. Pharm Biol. 2011;49(9):920-6. doi:10.3109/13880209.2011.556650

16. Adams RP. Identification of essential oil components by gas chromatography-quadrupole mass spectrometry. Allured Publishing Corporation. Carol Stream, IL; 2001. p.455.
17. Akbari V, Jamei R, Heidari R, Jahanban-ESfahlan A. Antiradical activity of different parts of Walnut (*Juglans regia* L.) fruit as a function of genotype. Food Chem. 2012;135(4):2404-10. doi:10.1016/j.foodchem.2012.07.030
18. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, et al. Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem. 1999;47(10):3954-62. doi:10.1021/jf990146l
19. Shin Y, Liu RH, Nock JF, Holliday D, Watkins CB. Temperature and relative humidity effects on quality, total ascorbic acid, phenolic and flavonoid concentrations, and antioxidant activity of strawberry. Postharvest Biol Technol. 2007;45(3):349-57. doi:10.1016/j.postharvbio.2007.03.007
20. Hatano T, Kagawa H, Yasuhara T, Okuda T. Two new flavonoids and other constituents in licore root: their relative astringency and radical scavenging affects. Chem Pharm Bull. 1988;36(6):2090-7. doi:10.1248/cpb.36.2090
21. Shafaghat A, Larijani K, Salimi F. Composition and antibacterial activity of the essential oil of *Chrysanthemum parthenium* flower from Iran. J Essent Oil Bear Plants. 2009;12(6):708-13. doi:10.1080/0972060x.2009.10643779
22. Singh P, Krishna A, Kumar V, Krishna S, Singh K, Gupta M, et al. Chemistry and biology of industrial crop Tagetes Species: a review. J Essent Oil Res. 2015;28(1):1-14. doi:10.1080/10412905.2015.1076740
23. Salamci E, Kordali R, Kotan A, Cakir A, Kaya Y. Chemical compositions, antimicrobial and herbicidal effects of essential oils isolated from Turkish *Tanacetum aucheranum and Tanacetum chiliophyllum var. chiliophyllum*. Biochem System Ecol. 2007;35(3):569-81. doi:10.1016/j.bse.2007.03.012
24. Mirjalili MH, Salehi P, Sonboli A, Vala M. Essential oil composition of Feverfew (*Tanacetum parthenium*) in wild and cultivated populations from Iran. Chem Nat Compd. 2007;43(2):218-20. doi:10.1007/s10600-007-0085-2
25. Izadi Z, Esna-Ashari M, Piri K, Davoodi P. Chemical composition and antimicrobial activity of feverfew (*Tanacetum parthenium*) essential oil. Int J Agric Biol. 2010;12(5):759-63.
26. Fasina OO, Hallman H, Craig-Schmidt M, Clements C. Predicting temperature-dependence viscosity of vegetable oils from fatty acid composition**.** J Am Oil Chem Soc. 2006;83(10):899-903. doi:10.1007/s11746-006-5044-8
27. Tsevegsuren N, Christie WW, Losel D. *Tanacetum* (*Chrysanthemum*) *corymbosum* seed oil a rich source of a novel conjugated acetylenic acid. Lipids. 1998;33(7):723-7. doi:10.1007/s11745-998-0262-2
28. Esmaeili MA, Sonboli A, Noushabadi MA. Antioxidant and protective properties of six *Tanacetum* species against hydrogen peroxide-induced oxidative stress in K562 cell line: A comparative study. Food Chem. 2010;121(1):148-55. doi:10.1016/j.foodchem.2009.12.022
29. Bariş D, Kizil M, Aytekin C, Kizil G, Yavuz M, Çeken B, et al. In vitro antimicrobial and antioxidant activity of ethanol extract of three Hypericum and three *Achillea* species from Turkey. Int J Food Prop. 2011;14(2):339-55. doi:10.1080/10942910903189256
30. Malekpoor F, Ghasemi A, Salimi S, Shabani L, Sharifi M, Hamedi B. Antimicrobial and antioxidant activities and total phenolic content of *Tanacenatum polycephalum* Schutz.Bip. as a folkloric herb in South western Iran. Indian J Tradit Know. 2015;14(3):370-5.
31. Wu C, Chen F, Wang X, Kim HJ, He GQ, Zitlin VH, et al. Antioxidant constituents in Feverfew (*Tanacetum parthenium*) extract and their chromatographic quantification. Food Chem. 2006;96(2):220-7. doi:10.1016/j.foodchem.2005.02.024
32. Firozy M, Talebpoura Z, Sonboli A. Essential oil composition and antioxidant activities of the various extracts of *Tanacetum sonbolii* Mozaff. (Asteraceae) from Iran. Nat Prod Res. 2012;26(23):2204-7. doi:10.1080/14786419.2011.636746
33. Polatoglua K, Karakoc OC, Goren N. Phytotoxic, DPPH scavenging, insecticidal activities and essential oil composition of *Achillea vermicularis*, *A .teretifolia* and proposed chemotypes of *A. biebersteinii* (Asteraceae). Ind Crops Prod. 2013;51:35-45. doi:10.1016/j.indcrop.2013.08.052
34. Miguel MG. Antioxidant and anti-inflammatory activities of essential oils: A short review. Molecules. 2010;15(12):9252-87. doi:10.3390/molecules15129252