Evaluation of the Phytochemical and Antioxidant Potential of Aerial Parts of Iranian Tanacetum parthenium
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A B S T R A C T

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-picrylhydrazyl (DPPH) radical scavenging technique.

Results: In the essential oil thirteen compounds were characterized with camphor (43.97 %), chrysantheneyl 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/chrysantheneyl 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.²³ 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 terpenoids, alkaloids and phenolic compounds which are considered as valuable basic constituents for food and pharmaceutical industries.²⁴ 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.³⁵ 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.³⁶⁷ Hence, phytochemical analysis of the plants is very important for nutritional and pharmaceutical applications.¹⁸ 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\textsuperscript{10} which have several therapeutic properties like as anti-septic, anti-microbial, anti-parasitic and anti-inflammatory properties.\textsuperscript{11,12} Also, it had been used as an insect repellent and had antioxidant, antifungal and antibacterial activity.\textsuperscript{12,13} Flavonoids, parthenolide and a number of related sesquiterpene lactones considered to be responsible for these activities.\textsuperscript{12} There are several reports on the volatile oil composition of \textit{T. parthenium}. In most cases, camphor (42-60\%), chrysanthenyl acetate (13-25\%), camphen (1.5-12\%) and \textit{p}-Cymene (0.1-5.2\%) are the main components together with various secondary components.\textsuperscript{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 \textit{T. parthenium} oil grown collected from Guilan, North of Iran.

Materials and Methods

\textbf{Collection of plant material}

Samples of the aerial parts of \textit{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.

\textbf{GC-MS analysis conditions}

The collected aerial parts (100g) were hydrodistilled for 3 h using a Cleveger-type system. Samples were dried with anhydrous sodium sulfate and kept in closed sterilized glass vials at 4°C 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).\textsuperscript{16}

\textbf{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 280°C and 250°C, 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 °C with the rate of 10 °C/min. For the identification of the compounds, retention times and retention index were confirmed with commercially available standard compounds (Sigma, Chemical Co.St. Louis).

\textbf{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 eusropher 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.\textsuperscript{17} Concentration of each individual compound was calculated using an external standard method and was converted to mg compound per g dry weight (dw).

\textbf{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.\textsuperscript{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\textsuperscript{2} =0.998 Eq.(1)).
Total flavonoid content
The total flavonoid content (TFC) of the essential oil was determined by the aluminium chloride colorimetric method. 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 °C, 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, R² = 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. 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:
% inhibition = [(AC − AS)/AC] × 100 Eq.(3)
where AC is the absorbance of control reaction and AS is the absorbance of the sample at 520 nm.

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. 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 sesquiterpenes fractions (33.15%). Differences can be clearly seen on the major constituents of T. parthenium volatile oil from the previous literature 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 components of the essential oil.
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.\textsuperscript{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 identified as 30.2\% and 26.5\%, respectively. Monoterpenoids were the main components of the oil (66.5\%), but sesquiterpenoids (20\%) had low percent.\textsuperscript{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 \%).\textsuperscript{21} Essential oils of aerial parts of the herb from Tehran province were investigated by Mirjalili et al.\textsuperscript{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.\textsuperscript{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\%), cycloisoolongifolene8-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 sesquiterpene can lead to a monoterpane amount decreases. However, the above mentioned studies display that these valuable metabolites are affected by both genetic and environmental factors.\textsuperscript{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.\textsuperscript{26}

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

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

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

**Phenolic (TPC) and flavonoid (TFC) contents**

The key role of phenolic and flavonoid components as antioxidants is emphasized in several reports. Therefore, it would be valuable to determine these antioxidants of the plant oil. To our knowledge, there are no published reports 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 (R² = 0.9988) was 152.2 ± 0.8 mg GAE/g dry plant sample (p < 0.05). Also The content of flavonoid compounds (R² = 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. reported that amount of total phenolic of essential oil of Tanacetum polycephalum varied from 0.063 to 0.153 (mg gallic acid g dw). In addition, Wu et al. 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. sonboli species different extracts was found that TFC varied from 1.5 to 41.3 µg Q Es/mg. 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, quantit planta 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.

**DPPH radical scavenging assay**

The DPPH test is widely used in assessing antioxidants because of the ease of the reaction. In our study, the antioxidant activity of essential oil of T. parthenium was expressed as IC₅₀ 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, when compared to the positive control Savsat oil (17.3%) showed low and Davutapa 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. The results suggest that the high scavenging activities of the oils are due to the content of monoterpene and sesquiterpene alcohols. 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.

<table>
<thead>
<tr>
<th>Phenolic compound</th>
<th>Calibration curve*</th>
<th>R²</th>
<th>Sample (mg/g DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rutin</td>
<td>Y=2011928x-1392956</td>
<td>0.998</td>
<td>-</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>Y=2372008.6x-1576952.1</td>
<td>0.997</td>
<td>-</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>Y=1278406.2x-1853153.7</td>
<td>0.988</td>
<td>-</td>
</tr>
<tr>
<td>4-Hydroxy benzoic acid</td>
<td>Y=762895x-733317</td>
<td>0.998</td>
<td>3.92 ± 0.3</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>Y=3159050.4x-296093</td>
<td>0.999</td>
<td>0.18 ± 0.1</td>
</tr>
<tr>
<td>P-coumaric acid</td>
<td>Y=82887x-59041</td>
<td>0.998</td>
<td>2.05 ± 0.2</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>Y=13571x-3682.9</td>
<td>0.985</td>
<td>1.96 ± 0.1</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>Y=165138x-136553</td>
<td>0.988</td>
<td>2.59 ± 0.1</td>
</tr>
<tr>
<td>Sinapic acid</td>
<td>Y=20727x-95900</td>
<td>0.977</td>
<td>3.86 ± 0.2</td>
</tr>
</tbody>
</table>

*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.

<table>
<thead>
<tr>
<th>Samples</th>
<th>TPC(mg GAE/g)</th>
<th>TFC(mg QUE/g)</th>
<th>DPPH(%)</th>
<th>IC₅₀(µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essential oil</td>
<td>152.2±0.8</td>
<td>70.2±0.3</td>
<td>73.8±1.3</td>
<td>30.23±0.8</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>-</td>
<td>-</td>
<td>93.12±0.4</td>
<td>0.15±0.0</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>-</td>
<td>-</td>
<td>92.30±0.2</td>
<td>0.16±0.0</td>
</tr>
</tbody>
</table>

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

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