

The biochemical contents and antioxidant activities of four *Tanacetum* L. taxa

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Abstract. The goal of the present study is to determine some of the biochemical compositions and antioxidant capacities of plant extracts in two endemic taxa for Turkey including *T. cadmeum* (Boiss.) Heywood subsp. *orientale* Grierson and *T. nitens* (Boiss. & Noë) Grierson together with *T. polycephalum* (L.) Sch.Bip subsp. *argyrophyllum* (K. Koch) Podlech, and *T. parthenium* (L.) Sch.Bip. The fatty acids were determined by using gas chromatography, while phenolics, lipid soluble vitamins and sterols were determined by using HPLC and radical scavenging activities, total phenolics, and FRAP were determined spectrophotometrically. It was found that *Tanacetum* taxa have palmitic acid (C16:0), and stearic acid (C18:0) as major saturated fatty acids and linoleic acid (C18:2 n6), α -linolenic acid (C18:3 n3) and oleic acid (C18:1 n9) as principal unsaturated fatty acids. It was found that *Tanacetum* taxa had more total unsaturated fatty acid contents (60.24 \pm 0.3%-70.54 \pm 0.29%) than saturated fatty acids and it was found that *T. parthenium* had the highest total essential fatty acid composition (58.65 \pm 0.59%). It was also reported that the omega6/omega3 ratio of *T. cadmium* subsp. *orientale* (8.22) differed from other taxa in this study. Also, the present study showed that *Tanacetum* had the lowest amount of lipid soluble vitamins. On the other hand, catechin was found to be the main polyphenolic compound in this study and it was determined that *T. parthenium* had the highest catechin (4479.1 \pm 5.71 μ g/mg) and total phenolic contents (324.91 \pm 2.01 μ gGAE/mg) in this study. Rutin was only determined in two endemic taxa *T. cadmeum* subsp. *orientale* (23 \pm 0.91 μ g/mg), and *T. nitens* (5.7 \pm 0.27 μ g/mg). Also, the naringenin, vanillic acid and caffeic acid amounts of the endemic *T. cadmeum* subsp. *orientale* and *T. nitens* were higher than other taxa in the study. In addition, it was determined that *Tanacetum* taxa had a high stigmasterol content. However, *T. parthenium* had a higher ergosterol content (271 \pm 2.36 μ g/mg). It was also found that *T. parthenium* has highest D2, α -tocopherol, retinol acetate, ergosterol, and stigmasterol contents among the studied taxa. In addition, the study showed that *Tanacetum* taxa have strong DPPH and ABTS radical scavenging activities. It was concluded that *Tanacetum* taxa have potent antioxidant capacity.

Keywords: Antioxidant capacity, fatty acids, lipid-soluble vitamins, phenolics, sterols, *Tanacetum* L.

Introduction

Tanacetum L., is one of the biggest genus of the Anthemideae in Asteracea, includes about 200 taxa, and spreads throughout Europe, North Africa, North America, and Asia (1,2). *Tanacetum* L. generally has perennial taxa and some of them are cultivated (3).

Also, the species of the genus are used as food spices and in cosmetics (4). The genus has 45 species and 60 taxa in Turkey flora, and the endemism ratio of the genus is 45% (5). The members of the genus are spread all over Turkey except for in the Aegean region (6).

Tanacetum L. species are known as “pireotu” in Turkish and they are used as medicinal plants in

traditional medicine (7,8). For example, *T. parthenium* is indexed in European Pharmacopeia because it was used in treatment of migraine as traditional medicine (8). And also, many *Tanacetum* species are known to have insect repellent properties due to their chemical content (6). In addition, several studies have shown that *Tanacetum* L. represent therapeutic effects such as anti-inflammatory, antiviral, anticancer, antibacterial, and antiulcer (9-11). Furthermore, they have been used in the cure of migraines, epilepsy, fever asthma, rheumatoid arthritis, stomach ache, and toothache (12,13). Also, they are used in the treatment of kidney disease, respiratory infections and abortivum as herbal cure (4).

It was reported that the genus has bioactive compounds such as essential oils, phenolics, terpenoid, and sterol compounds (9,13). And also, it was found that they had high antioxidant activity (8,11). There is interest in studies on *Tanacetum* due to its rich medicinal ingredients and being used in traditional medicine (8). However, some of the studies consist of essential oils, antimicrobial studies and cytotoxic studies of *Tanacetum* species other than those in these studies (7,10). The aim of the present study is to determine some of the biochemical contents (containing fatty acid compositions, lipid soluble vitamins, sterols), phenolics (flavonoids, phenolic acids, total phenolics) and antioxidant activities (radical scavenging capacity and ferric-reducing antioxidant power assay) of the plant extracts of two endemic taxa (*Tanacetum cadmeum* (Boiss.) subsp. *orientale* Grierson and *Tanacetum nitens* (Boiss. & Noë) Grierson) and *Tanacetum polycephalum* Schultz-Bip. subsp. *argyrophyllum* (K. Koch.) Podlech and *Tanacetum parthenium* (L.) Sch.Bip.. This study is thought to contribute to the determination of the lipid soluble vitamin content of *Tanacetum* because there is little knowledge in the literature.

Material and Methods

Plant materials

The plant materials were collected from natural habitats in 2010, and the samples were kept at Firat University Herbarium (FUH). The localities of the studied *Tanacetum* taxa are given in Table 1.

Table 1. Localities of *Tanacetum* L. taxa

Taxa	Locality
<i>Tanacetum cadmeum</i> (Boiss.) Heywood subsp. <i>orientale</i> Grierson, endemic	Malatya, Malatya-Puturge road, Kubbe gate, 1770 m.
<i>Tanacetum polycephalum</i> Sch. Bip. subsp. <i>argyrophyllum</i> (K.Koch) Podlech	Elazığ, Harput, Buzluk cave, 1565 m.
<i>Tanacetum parthenium</i> (L.) Sch.Bip.	Elazığ, Sivrice, Surek village, 1250-1300 m.
<i>Tanacetum nitens</i> (Boiss. & Noë) Grierson, endemic	Elazığ; Baskil, Hasan Mountain, Slope, 1950-2000 m

Extraction of the plant materials

The Analysis of fatty acid, lipid soluble vitamins, and sterols

2 g plant extracts were used hexane/isopropanol (2:3; v/v) in the detection of the fatty acid profile, lipid-soluble vitamins, and sterol (ergosterol, β -sitosterol and stigmasterol) amounts (14). Then, the lipid samples were centrifuged at 10.000 g for 5 minutes. Lastly, the solvent was removed with the aid of a rotary evaporator at 40 °C. The experiments were repeated three times, and the extracts were stored at -25°C.

The Analysis of the Fatty Acids

Fatty acids in the lipid extracts were converted into methyl esters by using 5 ml 2% sulphuric acid (v/v) in methanol and mixed by using vortex. This mixture is stored at 50 °C for 15 hours in the oven. The tubes were cooled to room temperature and mixed well by adding 5 ml of 5% sodium chloride. Then, the fatty acid methyl esters were extracted by using 5 ml n-hexane and hexane phase is extract with 5 ml 2% KHCO₃. And the samples were left for 4 hours to separate phases. Lastly, methyl esters are evaporated by using the nitrogen in 45 °C and the material was solved in 1 ml hexane and analysed in the gas chromatography (15). A capillary column was used (25 m in length and 0.25 mm in diameter; Perma-bound 25, Macherey-Nagel, Germany) and Shimadzu GC 17 gas chromatography was used to separate the methyl ester profiles of the *Tanacetum* taxa. The nitrogen was used as carrier gas and % fatty acid results were

given. The heat of the detector, column, and injection valve were 240, 130-220 and 280 °C, respectively.

Chromatographic analysis and quantification of the lipid soluble vitamins and sterols

The lipid soluble vitamins and sterol (ergosterol, stigmasterol and β -sitosterol) contents were determined according to the Sánchez-Machado et al. (16)' method. The extracts were extracted with acetonitril/methanol (75/25 v/v) and A Supelcosil TM LC18 (250 x 4.6 mm, 5 mm, Sigma, USA) were used as the column in the HPLC ((Shimadzu, Japan). The determination wavelength of the retinol and retinol acetate were conducted at 320 nm, while the wavelength of the tocopherol, vitamin D, α -tocopherol, and α -tocopherol acetate were 215 nm and the wavelength of vitamin K1 and K2 were 265 nm, and the wavelength of phytosterols were 202 nm (17). Class Vp 6.1 software was used to determine the amounts in the study and the results are given as $\mu\text{g/g}$.

Chromatographic Conditions for Phenolics

The plant extracts were treated with 5 ml 80 % methanol and the examples were centrifuged at 5000 rpm at +4°C. Dimethyl sulphoxide (DMSO) was used as a stock solution. The column was the PREVAIL C18 reversed-phase (15x4.6mm, 5 μm , USA) and the mobile phase was methanol /water/acetonitrile (46/46/8, v/v/v) containing 1.0% acetic acid (18). The flow ratio was 1.0 ml/min. and the chromatographic peaks were verified by determining the retention times with those of the standards. Rutin, morin, quercetin, myricetin, and vanilic acid at 254 nm, catechin, naringin and cinnamic acid at 280 nm; naringenin at 285 nm; kaempferol at 264 nm, resveratrol at 306 nm and ferulic acid, caffeic acid and rosmarinic acid at 330 nm were determined by DAD following the RP-HPLC.

Antioxidant activity

DPPH radical scavenging capacity

The DPPH radical scavenging activity was determined according to the Liyana- Pathiranan and Shahidi (19)' method. The complex was left in the dark for

30 minutes at room temperature after 4.0 ml DPPH solution was mixed with 25, 50, 100, 150. and 250 μL of extract. The absorbances were measured in spectrophotometer (Shimadzu UVmini-1240, UV-Vis Spectrophotometer) at 517 nm. 1 μM quercetin was used as a reference value (19). The results were determined by using formula:

$$\text{DPPH radical scavenging capacity (\%)} = [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})] / (\text{Abs}_{\text{control}})] \times 100$$

An Abs_control is the absorbance of the DPPH radical + methanol; an Abs_sample is the absorbance of DPPH radical + sample extract/standard.

ABTS 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) Diammonium Salt Assay

The ABTS radical cation capacity was calculated based on the method of Ree *et al.* (20). The ABTS radical cation (ABTS \bullet^+) and 7 mM ABTS mixed with 2,45 mM potassium persulphate were used to obtain the ABTS radical cation. The sample solutions were kept for 12–16 h at room temperature. Then the (ABTS \bullet^+) solution was dissolved with water to measure an absorbance of 0.700 ± 0.020 at 734 nm and the absorbances of samples were measured in spectrophotometer (Shimadzu UVmini-1240, UV-Vis Spectrophotometer). The 3 ml ABTS solution was extracted with 25, 50, 100, 150, and 250 μL samples, and the absorption was detected during 6 min. The absorbance of the control (3.0 mL (ABTS \bullet^+) solution with 30 L water) was written as Acontrol. (21).

$$\text{The ABTS radical cation scavenging capacity (\%)} = [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})] / (\text{Abs}_{\text{control}})] \times 100.$$

Determination of the Total Phenolics

The total phenolic contents were determined according to the Folin–Ciocalteu method (22). The 100 μl samples were added to the mixture containing 200 μl of Folin–Ciocalteu reagent and 3.16 ml of H₂O. The extracts were kept at room temperature for two hours after the anhydrous sodium carbonate (20%, w/v) was added to the mixture. Then, the samples were kept at room temperature for 3 min. The absorbance of samples was detected at 765 nm in spectrophotometer (Shimadzu UVmini-1240, UV-Vis Spectrophotometer) (23). The

total phenolic amount was calculated by using the gallic acid equivalents per gram dry weight ($\mu\text{gGAE}/\text{mg}$).

Ferric-reducing antioxidant power assay (FRAP)

The FRAP method was conducted by using the protocol of the Benzie and Strain (24) method. Sodium acetate buffer (300 mmol/l), TPTZ (2,4,6-tripyridyl-striazine) solution in 40 mmol/l and 20 mmol/l FeCl_3 (10:1:1; v/v) have been used to obtain the FRAP solution. The absorbance has been measured at 593 nm after the 10 min. FeSO_4 solution (100–2000 mmol/L) is used to form the standard curve.

Results and Discussion

*The fatty acid compositions, lipid-soluble vitamin and sterol contents of *Tanacetum L. taxa**

The data obtained from the present study showed that palmitic acid (16:0) was a major saturated fatty acid ($12.63\pm 0.34\%$ – $21.76\pm 0.56\%$) and stearic acid (18:0) was the second major saturated fatty acid ($2.86\pm 0.15\%$ – $5.63\pm 0.11\%$) (Table 2). *Tanacetum nitens* had the highest palmitic acid amounts ($21.76\pm 0.56\%$), while *Tanacetum cadmeum* subsp. *orientale* had the lowest ($12.63\pm 0.34\%$). On the contrary, Rezaei *et al.* (25)

Table 2. Fatty acid compositions of *Tanacetum L. taxa* (%)

Fatty Acids	<i>T. cadmeum</i> subsp. <i>orientale</i>	<i>T. polycephalum</i> subsp. <i>argyrophyllum</i>	<i>T. parthenium</i>	<i>T. nitens</i>
10:0	8.28±0.34	0.88±0.1	6.74±0,26	3.69±016
11:0	-	-	0.36±0,05	-
12:0	0.85±0.01	6.01±0.24	-	-
14:0	1.59±0.1	2.17±0.11	0.81±0,07	-
16:0	12.63±0.34	18.78±0.54	12.46±0,96	21.76±0.56
17:0	-	-	1.32±0.14	-
18:0	3.09±0.12	2.86±0.15	3.27±0.21	5,63±0.11
20:0	3,79±0.15	3.91±0.21	1.17±0.1	-
23:0	4.32±0.23	5.09±0.32	-	-
24:0	-	-	1.44±0.12	-
ΣSaturated fatty acids	34.55±0.18	39.7±0.27	26.13±0.23	31.08±0.27
14:1	-	-	0.72±0.04	-
16:1 n7	1.62±0.1	2.93±0.11	3.87±0.27	-
18:1 n9	7.69±0.34	4.03±0.1	6.3± 0.42	15.14±0.14
18:2 n6c	41.36±0.89	18.22±0.32	20.41±0.76	17.19±0.22
18:3n3	5.03±0.21	27.74±0.78	38.24±0.43	36.57±0.35
20:4n3	3.19±0.13	3.88±0.29	1.00±0.04	-
20:1 n9	-	-	1.77±0.08	-
24:1	6.45±0.22	3.44±0.25	-	-
ω -6/ ω -3	8.22	0.65	0.53	0.47
ΣEssential fatty acids	46.39±0.55	45.96±0.55	58.65±0.59	53,76±0.29
ΣMonounsaturated fatty acids	15.76±0.48	10,4±0.15	12,66±0,2	15.14±0.14
ΣPolyunsaturated fatty acids	49.58±0,41	49.84±0.46	59.65±0.41	53.76±0.28
ΣUnsaturated fatty acids	65.34±0.45	60.24±0.3	70.31±0.3	68.9±0.21

discovered that the major saturated fatty acids of *Tanacetum* are palmitic acid (16:0) and myristic acid (14:0). Besides, *Tanacetum polycephalum* subsp. *argyrophyllum* had the highest saturated fatty acid content ($39.7 \pm 0.27\%$) whilst *Tanacetum parthenium* had lowest saturated fatty acid content ($26.13 \pm 0.23\%$). Also, capric acid (10:0), myristic acid (C14:0), arachidic acid (20:0), and tricosylic acid (23:0) were found in the *Tanacetum* taxa (Table 2). This study demonstrated that linoleic acid (18:2 n6), α -linolenic acid (18:3 n3), and oleic acid (18:1 n9) are major unsaturated fatty acids in the study of four *Tanacetum* taxa (Table 2). The linoleic acid content was found between $17.19 \pm 0.22\%$ (*Tanacetum nitens*) and $41.36 \pm 0.89\%$ (*Tanacetum cadmeum* subsp. *orientale*), while α -linolenic acid was found between $5.03 \pm 0.21\%$ (*Tanacetum cadmeum* subsp. *orientale*) and $38.24 \pm 0.43\%$ (*Tanacetum parthenium*) and also the oleic acid content was found between $4.03 \pm 0.1\%$ (*Tanacetum polycephalum* subsp. *argyrophyllum*) and $15.14 \pm 0.14\%$ (*Tanacetum nitens*). Furthermore, it was determined that *Tanacetum parthenium* had the highest total and poly unsaturated fatty acid composition ($70.54 \pm 0.29\%$; $58.65 \pm 0.59\%$) in this study. On the contrary, Rezaei *et al.* (25) determined that the linoleic acid, α -linolenic acid and oleic acid contents of the *Tanacetum parthenium* were low (6.03%, 2.03% and 0.01%, respectively). They indicated that *Tanacetum parthenium* had the highest palmitic acid (57.27%) and saturated fatty acid (87.96%) content (25). Ayaz *et al.* (26) found that the unsaturated fatty acid composition of the Anthemideae was about 69.39% and they indicated that linoleic acid is the principal unsaturated fatty acid in Anthemideae. Also, they determined that the polyunsaturated fatty acid content of the *Tanacetum* species was 72.89% (26). Similarly, Tonguc and Erbas (27) showed that linoleic acid (15.24%–66.22%) was found as polyunsaturated fatty acid in Asteraceae but linolenic acid was not detected. And also, Arslan and Tarıkahya Hacıoğlu (28) mostly found linoleic acid (58.8%–82.6%) as polyunsaturated fatty acid in Asteraceae. In addition, Özcan *et al.* (29) showed that linoleic acid (C18:2 n6) had the highest amounts (52.1%–75.2%) and the linolenic acid showed significant variation (0.5%–17.3%) in Asteraceae. ω -3 (α -linolenic acid) and ω -6 (linoleic acid) fatty acids are known as essential fatty acids. They cannot be synthesized by humans and so must be absorbed from

dietary intake (30). It has been suggested that there is a positive correlation between essential fatty acids and the reduction of several chronic illnesses such as cardiovascular disease, cancer, diabetes, and neurological disease (31). This study showed that *Tanacetum cadmium* subsp. *orientale* had the lowest ω -3 content ($5.03\% \pm 0.21\%$) and the highest ω -6 content ($41.36\% \pm 0.89\%$) compared to the other studied *Tanacetum* taxa (Table 2). The total amounts of essential fatty acids of *Tanacetum parthenium* ($58.65 \pm 0.59\%$) and *Tanacetum nitens* ($53.76 \pm 0.29\%$) taxa were found to be higher than *Tanacetum cadmium* subsp. *orientale* ($46.39 \pm 0.55\%$) and *Tanacetum polycephalum* subsp. *argyrophyllum* ($45.96 \pm 0.55\%$). Furthermore, linoleic acid and linolenic acids are used as biochemical tools to solve systematic problems (32). It was found that the ω 6/ ω 3 ratio of *Tanacetum cadmium* subsp. *orientale* (8.22) differed from *Tanacetum polycephalum* subsp. *argyrophyllum* (0.65), *Tanacetum parthenium* (0.53) and *T. nitens* (0.47). Palmitoleic acid (C16:1 n7) was found to be as the other unsaturated fatty acid in all the taxa studied (Table 2).

On the other hand, this study demonstrated that the *Tanacetum* species had low lipid soluble vitamin contents (Table 3). Phytosterols comprise over 250 different sterols and play significant roles in fighting cancer and cardiovascular disease (33). Sitosterol, campesterol and stigmasterol are rich in plant sterols that cannot be synthesized by humans (34,35). They are mostly used as cholesterol-reducing supplements in foods and play a significant role in the plasma membrane, the outer membrane of the endoplasmic reticulum, and the mitochondria. They are a defining feature of these membranes (35,36). It was found that the stigmasterol content of the studied *Tanacetum* taxa was relatively high (72.85 ± 1.12 $\mu\text{g/g}$ – 315.5 ± 2.56 $\mu\text{g/g}$) and *Tanacetum parthenium* had the highest ergosterol (271 ± 2.36 $\mu\text{g/g}$) and stigmasterol (315.5 ± 2.56 $\mu\text{g/g}$) contents in the present study. The β -sitosterol contents of *Tanacetum parthenium* and *Tanacetum nitens* were either exceptionally low or entirely absent (Table 3). Azizudin and Choudhary (37) found that β -sitosterol and stigmasterol were found in the *Tanacetum polycephalum* while Ivanescu Tuchilus *et al.* (11) reported that *Tanacetum* species included β -sitosterol, traces of ergosterol, and stigmasterol content. This study may be the first work on the lipid-soluble vitamin content of the studied *Tanacetum* taxa.

Table 3. Lipid soluble vitamin and sterol contents of *Tanacetum* L. taxa ($\mu\text{g/g}$)

Lipid-soluble vitamins	<i>T. cadmeum</i> subsp. <i>orientale</i>	<i>T. polycephalum</i> subsp. <i>argyrophyllum</i>	<i>T. parthenium</i>	<i>T. nitens</i>
K1	1.7 \pm 0.1	0.4 \pm 0.01	2.6 \pm 0.11	3.55 \pm 0.13
K2	0.45 \pm 0.01	1.4 \pm 0.1	-	0.45 \pm 0.01
R-tocopherol	3.15 \pm 0.11	0.05 \pm 0.001	6.94 \pm 0.14	0.65 \pm 0.01
D2	0.8 \pm 0.01	0.4 \pm 0.001	12.4 \pm 0.45	1.8 \pm 0.1
D3	0.05 \pm 0.001	0.05 \pm 0.001	0.15 \pm 0.01	0.6 \pm 0.001
a-tocopherol	7.35 \pm 0.11	8.05 \pm 0.15	83.4 \pm 0.89	8.25 \pm 0.13
Retinol	-	-	-	-
Retino acetate	0.1 \pm 0.01	1.7 \pm 0.1	9 \pm 0.19	3.55 \pm 0.14
Ergosterol	1.75 \pm 0.012	2 \pm 0.1	271 \pm 2.36	0.2 \pm 0.01
Stigmasterol	72.85 \pm 1.12	127.9 \pm 1.27	315.5 \pm 2.56	129.55 \pm 2.48
B-sitosterol	60.2 \pm 1.11	26.2 \pm 0.54	-	0.2 \pm 0.01

The phenolic contents of *Tanacetum* L. taxa

Medicinal plants have significant roles in the pharmaceutical industry because they are used as therapeutic agents and raw materials in alternative and modern medicines (38). In addition, there is great interest in natural antioxidants because synthetic antioxidants have long-term toxicological side effects (39). Polyphenols have significant antioxidant activities and play major roles in fighting oxidation, carcinogenesis, coronary heart disease, and inflammation (40,41). In this study, it was reported that *Tanacetum parthenium* had the highest catechin amounts (4479.1 \pm 5.71 $\mu\text{g/mg}$) while *Tanacetum cadmeum* subsp. *orientale* had lowest catechin amount (1011.9 \pm 3.3 $\mu\text{g/mg}$; Table 4). Catechins, known as tea polyphenolics, play major roles in scavenging free radicals, inhibiting oxidative enzymes, and suppressing cancer-related transcriptional factors so there is increasing interest in them (42). Although it has been reported that kaempferol glycosides are the chief phenolics in Asteraceae (43), this study showed that catechin is the main phenolic component in the *Tanacetum*. Similarly, Gecibesler *et al.* (44) showed that catechin is the principal component in *Tanacetum*. Also, rutin has been found in the endemic taxa comprising *Tanacetum cadmeum* subsp. *orientale* (23 \pm 0.91 $\mu\text{g/mg}$) and *Tanacetum nitens* (5.7 \pm 0.27 $\mu\text{g/mg}$) while naringenin was found at the highest amounts in *Tanacetum cadmeum* subsp. *orientale* (72.1 \pm 0.81 $\mu\text{g/mg}$) and

Tanacetum nitens (108.4 \pm 1.26 $\mu\text{g/mg}$). Naringenin is low or absent in the studied *Tanacetum* taxa (Table 4). In a study done by Zengin *et al.* (45) demonstrated there were different phenolics found in *Tanacetum* but a lot of them are small amounts. On the other hand, this study showed that the vanillic acid content of the *Tanacetum cadmeum* subsp. *orientale* (121 \pm 1.21 $\mu\text{g/mg}$), *Tanacetum polycephalum* subsp. *argyrophyllum* (228.2 \pm 1.34 $\mu\text{g/mg}$) and *Tanacetum nitens* (208.8 \pm 2.14 $\mu\text{g/mg}$) were relatively high (Table 4). Also, this study found that the caffeic acid content of two endemic taxa *Tanacetum cadmeum* subsp. *orientale* and *Tanacetum nitens* were 54.2 \pm 1.27 $\mu\text{g/mg}$ and 208.8 \pm 2.14 $\mu\text{g/mg}$, respectively, while the ferulic acid content of the *Tanacetum polycephalum* subsp. *argyrophyllum* was 69.0 \pm 1.14 $\mu\text{g/mg}$. However, based on the results of the current study, it was found that the cinnamic acid and the rosmarinic acid amounts were the lowest or absent. (Table 4). Savci *et al.* (46) found that *Tanacetum* taxa had myricetin (2843.3-5169.3 $\mu\text{g/ml}$), quercetin (5687.2-159698.5 $\mu\text{g/ml}$), kaempferol (3608.5-5470.6 $\mu\text{g/ml}$), caffeic acid (1504.8-12438.6 $\mu\text{g/ml}$), cinnamic acid (2771.2-3565.6 $\mu\text{g/ml}$), and rosmarinic acid (3143.0-3875.8 $\mu\text{g/ml}$). Moreover, Esmaeili *et al.* (47) indicated that *Tanacetum* taxa had caffeic acid, ferulic acid, luteolin, apigenin, and rutin as major phenolic compounds in six *Tanacetum* species, except for in this study. Also, previous studies have shown that the *Tanacetum* species have quercetin, apigenin, luteolin, chlorogenic

Table 4. Flavonoid ($\mu\text{g}/\text{mg}$) and phenolic acids ($\mu\text{g}/\text{mg}$) of *Tanacetum* L. taxa

	Phenolics	<i>T. cadmeum</i> subsp. <i>orientale</i>	<i>T. polycephalum</i> subsp. <i>argyrophyllum</i>	<i>T. parthenium</i>	<i>T. nitens</i>
Flavonoids	Rutin	23 \pm 0.91	-	-	5.7 \pm 0.27
	Myricetin	4.2 \pm 0.24	1.1 \pm 0.1	1.5 \pm 0.1	18.5 \pm 0.79
	Quercetin	31.4 \pm 0.78	47.5 \pm 0.84	3.1 \pm 0.24	-
	Kaempferol	0.8 \pm 0.01	-	7.4 \pm 0.35	18.8 \pm 0.75
	Catechin	1011.9 \pm 3.39	1451.4 \pm 3.42	4479.1 \pm 5.71	1865.3 \pm 4.79
	Naringin	-	0.2 \pm 0.01	-	1.2 \pm 0.34
	Naringenin	72.1 \pm 0.81	17.8 \pm 0.56	8.9 \pm 0.12	108.4 \pm 1.26
	Resveratrol	-	-	-	-
Phenolic Acids	Vanillic acid	121 \pm 1.21	228.2 \pm 1.34	6 \pm 0.14	208.8 \pm 2.14
	Cinnamic acid	0.2 \pm 0.01	-	-	-
	Caffeic acid	54.2 \pm 1.27	2.4 \pm 0.52	4.8 \pm 0.71	122.8 \pm 1.25
	Ferulic acid	14.6 \pm 0.77	69 \pm 1.14	-	16 \pm 0.45
	Rosmarinic acid	-	6 \pm 0.74	-	3 \pm 0.12

acid, kaempferol, rosmarinic acid, ferulic acid, cichoric acid, and rosmarinic acid (8,48). Besides, it has been reported that quantitative and qualitative differences in phenolic content can be accepted as taxonomical markers in the tribes and subtribes of Asteraceae (49,50). This study showed that the phenolic contents and their amounts differ in the studied *Tanacetum* taxa.

Antioxidant capacities of the *Tanacetum* L. taxa

The DPPH results demonstrated that the studied *Tanacetum* taxa had high radical scavenging capacities except for *Tanacetum cadmeum* (62.66 \pm 0.74%) and *Tanacetum nitens* (61.5 \pm 0.75 %), which had the lowest DPPH radical scavenging capacity in 25 μl (Table 5). However, *Tanacetum parthenium* and *Tanacetum nitens* had the lowest DPPH radical scavenging activities (74.02 \pm 0.72 % and 76.29 \pm 0.75 %, respectively) in 1000 μl . Furthermore, all of the studied taxa had over 85% of DPPH radical scavenging capacity in 250 μl and 500 μl (Table 5). Also, this study determined that all of the studied *Tanacetum* taxa had the highest ABTS radical scavenging activity (Table 5). It was found the *Tanacetum* taxa had the highest ABTS radical scavenging activities in 150 μl (91.72 \pm 0.57 %-93.79 \pm 0.98%), 250 μl (97.93 \pm 0.94%-99.31 \pm 1.11%) and 500 μl (97.75 \pm 0.65 %-99.97 \pm 1.11%). The results of this study demonstrated that the ABTS

radical scavenging activity of the *Tanacetum* taxa were under 90% in 100 μl except for in the *Tanacetum cadmeum* (92.75 \pm 0.74 %) (Table 5). This study demonstrated that *Tanacetum* taxa had high DPPH and ABTS radical scavenging activities (Tables 5). The results of the present study agreed with Baczeka *et al.* (8). They determined that *Tanacetum* taxa had strong DPPH and FRAP activity (8). Similarly, Arituluk *et al.* (51) and Yur *et al.* (52) found that *Tanacetum* taxa had strong DPPH activity. Also, Prashanth *et al.* (53) indicated that the maximum DPPH and ABTS radical scavenging capacities of *Tanacetum parthenium* were 67.90% and 87.10%, respectively. Besides, it was determined that *Tanacetum parthenium* had the highest total phenolic content (324.91 \pm 2.01 μg GAE/mg) and FRAP activity (2176 \pm 5.72 mM Fe (II)/g) in the present study (Table 5). Also, Tepe and Sokmen (54) found that the total phenolic contents of the *Tanacetum* taxa were between 146.14 \pm 1.79 mg/g and 162.33 \pm 3.57 mg/g. Another study conducted by Erdogan and Baydas (48) demonstrated the *Tanacetum* species had strong radical scavenging activity and reducing power capacity. However, Esmaeili *et al.* (47) found the total phenolic content of the six *Tanacetum* taxa was low (32.15 \pm 1.15 mg/g-47.11 mg/g). Servi *et al.* (55) indicated that soil structure, ecological factors, genotypes and climatic conditions lead to the differences in the biochemical contents of species.

Table 5. The DPPH %, ABTS%, total phenolics ($\mu\text{gGAE}/\text{mg}$) and FRAP amounts ($\text{mM Fe (II)}/\text{g}$) of *Tanacetum* taxa

	<i>T. cadmeum</i> subsp. <i>orientale</i>	<i>T. polycephalum</i> subsp. <i>argyrophyllum</i>	<i>T. parthenium</i>	<i>T. nitens</i>	
DPPH	25 μl	62.66 \pm 0.74	91.5 \pm 0.97	90.9 \pm 0.81	61.5 \pm 0.75
	50 μl	88.63 \pm 0.77	87.3 \pm 0.84	79.38 \pm 0.71	88.9 \pm 0.98
	100 μl	91.72 \pm 0.78	90.25 \pm 0.69	88.96 \pm 0.93	88.96 \pm 0.91
	150 μl	94.80 \pm 0.92	93.01 \pm 0.86	91.39 \pm 0.79	92.2 \pm 0.79
	250 μl	97.56 \pm 1.21	94.64 \pm 0.97	91.72 \pm 0.79	91.88 \pm 0.85
	500 μl	93.50 \pm 1.12	88.79 \pm 0.88	85.06 \pm 0.56	89.44 \pm 0.91
	1000 μl	91.88 \pm 1.01	81.00 \pm 0.89	74.02 \pm 0.72	76.29 \pm 0.75
	ABTS	25 μl	83.05 \pm 1.01	99.36 \pm 1.12	98.1 \pm 1.14
50 μl		91.67 \pm 0.83	93.79 \pm 0.91	92.75 \pm 1.21	88.1 \pm 0.79
100 μl		92.75 \pm 0.74	88.44 \pm 0.61	83.1 \pm 0.56	86.55 \pm 0.88
150 μl		93.79 \pm 0.98	93.22 \pm 0.77	91.72 \pm 0.57	93.1 \pm 0.93
250 μl		98.77 \pm 1.13	99.31 \pm 1.11	97.93 \pm 0.94	98.79 \pm 0.89
500 μl		99.02 \pm 0.94	98.79 \pm 0.93	97.75 \pm 0.65	97.93 \pm 0.76
Total phenolics	198.94 \pm 1.91	293.85 \pm 1.84	324.91 \pm 2.01	229.031 \pm 2.13	
FRAP	1906 \pm 4.52	1958.5 \pm 5.17	2176 \pm 5.72	1888.51 \pm 6.27	

Conclusion

This study showed that palmitic acid, stearic acid, linoleic acid, α -linolenic acid and oleic acid are principal fatty acids of the *Tanacetum* taxa. This study demonstrated that *Tanacetum* taxa had more total unsaturated fatty acid contents (60.24 \pm 0.3%-70.54 \pm 0.29%) than saturated fatty acids. In addition, *Tanacetum parthenium* had the highest total essential fatty acid composition (58.65 \pm 0.59%). It was also found that the ω -6/ ω -3 ratio of *Tanacetum cadmeum* subsp. *orientale* (8.22) differed from other taxa in this study. Also, it demonstrated that the lipid soluble vitamin content of *Tanacetum* species were low, In addition, catechin was found to be the major phenolic in the studied *Tanacetum* taxa and these results demonstrated that *Tanacetum parthenium* had the highest catechin and total phenolic contents. Rutin was only found in two endemic taxa *Tanacetum cadmeum* subsp. *orientale* (23 \pm 0.91 $\mu\text{g}/\text{mg}$), and *Tanacetum nitens* (5.7 \pm 0.27 $\mu\text{g}/\text{mg}$). Also, the amounts of naringenin, vanillic acid and caffeic acid found in the endemic *Tanacetum cadmeum* subsp. *orientale* and *Tanacetum nitens* were higher than those of the other taxa in the study. In addition, it was determined that *Tanacetum*

taxa had a high stigmaterol content. Moreover, this study suggests that *Tanacetum* taxa have significant radical scavenging and reducing power activity.

Conflicts of Interest

The authors declare that no conflicts of interest exist.

**Some of data in this study are presented in 2nd Int. Congress on Applied Biological Science and 23rd National Biology Congress as a poster and oral representation.

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