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Original Article

The biochemical content and antioxidant capacities of endemic *Tanacetum densum* (Lab.) Schultz Bip. subsp. *laxum,* and *Tanacetum densum* (Lab.) Schultz Bip. subsp. *amani* Heywood growing in Turkey

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Abstract

Medicinal plants have a significant role in preventing and curing several diseases, and *Tanacetum* L. is one of these plants. The aim of the present study is to determine the fatty acid, lipid-soluble vitamin, sterol, phenolic content, and antioxidant capacity of *Tanacetum densum* subsp. *laxum* and *Tanacetum densum* subsp. *amani,* to compare the effect of altitude on the biochemical content and to compare systematically by using fatty acids and phenolics. This study showed that palmitic acid (C16:0) and stearic acid (C18:0) are major sources of saturated fatty acid and oleic acid (C18:1 n9), and linoleic acid (18:2 n6c) and a-linolenic acid (C18:3 n3) are the principal unsaturated fatty acids in the two endemic *Tanacetum densum* taxa. Also, this study found that the unsaturated fatty acid content (60.11±1.61%) of *Tanacetum densum* subsp. *laxum* was higher than the unsaturated fatty acid content (44.13±1.28%) of *Tanacetum densum* subsp. *amani*. And also, the ω6/ω3 ratio of *Tanacetum densum* subsp. *laxum* (1.74) and *Tanacetum densum* subsp. *amani* (1.60) was found to be similar. However, this study determined that the lipid soluble vitamin and sterol content of two endemic *Tanacetum* taxa are low except for stigmasterol. Present study showed that catechin is principal phenolic in the *Tanacetum densum* taxa. This study also found that *Tanacetum densum* subsp. *laxum* and *Tanacetum densum* subsp. *amani* had the highest levels of catechin, vanillic acid, and caffeic acid content though the phenolic amounts, particularly catechin and quercetin, were dissimilar in the *T. densum* taxa. This study suggested that ecological conditions such as altitude may affect the biochemical content of two endemic *Tanacetum densum* taxa. Furthermore, the current study determined that two endemic *Tanacetum* L. taxa had potent radical scavenging capacities and found a correlation between total phenolics and antioxidant activity.

Keywords: antioxidant capacity, fatty acids, lipid soluble vitamins, sterols, *Tanacetum densum*.

O conteúdo bioquímico e as capacidades antioxidantes de *Tanacetum densum* endêmico (lab.) Schultz Bip. subsp. *laxum* e *Tanacetum densum* (lab.) Schultz Bip. subsp. *amani* Heywood que crescem na Turquia

Resumo

As plantas medicinais têm um papel significativo na prevenção e cura de várias doenças, e *Tanacetum* L. é uma dessas plantas. O objetivo do presente estudo é determinar o conteúdo de ácido graxo, vitamina lipossolúvel, esterol, estrutura fenólica e capacidade antioxidante de *Tanacetum densum* subsp. laxum e *Tanacetum densum* subsp. *amani*, comparar o efeito da altitude sobre o conteúdo bioquímico e realizar uma comparação sistemática usando ácidos graxos e fenólicos. Esta pesquisa mostrou que o ácido palmítico (C16:0) e o ácido esteárico (C18:0) são as principais fontes de ácido graxo saturado e que o ácido oleico (C18:1 n9), o ácido linoleico (18:2 n6c) e o ácido a-linolênico (C18: 3 n3) são os principais ácidos graxos insaturados nos dois táxons endêmicos de *Tanacetum densum*. Além disso, este estudo descobriu que o conteúdo de ácidos graxos insaturados (60,11±1,61%) de *Tanacetum densum* subsp. *laxum* foi superior ao conteúdo de ácidos graxos insaturados (44,13±1,28%) de *Tanacetum densum* subsp. *amani*, e também que a razão ω6/ω3 de *Tanacetum densum* subsp. *laxum* (1,74) e *Tanacetum densum* subsp. *amani* (1,60) foi semelhante. No entanto, este trabalho determinou que o conteúdo de vitamina lipossolúvel e esterol de dois táxons endêmicos de *Tanacetum* é baixo, exceto o estigmasterol, além de descobrir que *Tanacetum densum* subsp. *laxum* e *Tanacetum densum* subsp. *amani* apresentaram os mais altos níveis de conteúdo de catequina, ácido vanílico e ácido cafeico, embora as quantidades fenólicas, especialmente catequina e quercetina, sejam diferentes nos táxons de *T. densum*. Este estudo sugere que condições ecológicas, como a altitude, podem afetar o conteúdo bioquímico de dois táxons endêmicos de *Tanacetum densum*. Ainda, esta pesquisa determinou que dois táxons de *Tanacetum* L. endêmicos possuíam potentes capacidades de sequestro de radicais e que houve correlação entre fenólicos totais e atividade antioxidante.

Palavras-chave: capacidade antioxidante, ácidos graxos, vitaminas lipossolúveis, esteróis, *Tanacetum densum*.

1. Introduction

The Asteraceae family includes about 23.000 species throughout the world, and *Tanacetum* L. is the third biggest genus in the family (Yur et al., 2017). *Tanacetum* L. is found extensively in the northern hemisphere, especially in Europe, western Asia, North America, and north Africa include approximately 200 species (Oberprieler et al., 2007; Baranauskienė et al., 2014; Korkmaz et al., 2015; Bączek et al., 2017). The members of the genus generally are perennial and vary from herbs to subshrubs (Oberprieler et al., 2007).

Tanacetum L. has significant biological effects including antimicrobial, antioxidant, anti-inflammatory, and anticancer, and the genus has been used in folk medicine since ancient times for the treatment of migraine, stomach ache, toothache, insect bites, cancer, ulcers, high fever, arthritis, and vertigo (Marete et al., 2009; Marzouk et al., 2016; Mot et al., 2018; Coban et al., 2019). Additionally, *Tanacetum*, which is grown in gardens, is used in cosmetics and as a spicy food additive (Maxia et al., 2015; Mot et al., 2018). It has been demonstrated that essential oils, sterols, flavonoids, and sesquiterpenes are commonly found in this genus (Kilic, 2014; Marzouk et al., 2016). The genus is represented in Turkey by sixty taxa, and the endemism ratio is 43% (Orhan et al., 2015). *T. densum*, one of the endemic species, has four subspecies growing in Turkey: subsp. *sivasicum*, subsp. *laxum*, subsp. *amani* and subsp. *eginense* (Goren et al., 1995). *Tanacetum densum* is perennial, endemic, erect, or ascending subshrubs, the habitat is the limestone rocks and screes (1500-2500), and flowering time period is between June and August (Davis, 1988). The aim of the present study was to analyze the fatty acids, lipid-soluble vitamins, sterols, total phenolics, flavonoids, and phenolic acids as well as the radical scavenging and FRAP activities of plant extracts of endemic *T. densum* subsp. *laxum* and *T. densum* subsp. a*mani* in order to contribute to knowledge of the medicinal properties of two *Tanacetum* taxa. Another aim of this study was to examine the effect of altitude on fatty acid and phenolic content and to systematically evaluate the fatty acid composition and phenolic content.

2. Material and Methods

All chemicals were purchased from Sigma-Aldrich. The plant materials were collected from natural habitats in 2014 July, and samples were stored at the Firat University Herbarium (FUH). The localities of the two taxa under examination are given Table 1.

2.1. Extraction of plant materials

2.1.1. Analysis for fatty acids, lipid soluble vitamins, and sterols

Two g of plant materials was ground in a mill, and isopropanol/hexane (2:3 v/v) was added to analyze fatty acid, sterol, and lipid soluble vitamins (Hara & Radin, 1978). The lipid extract was centrifuged at 10.000 g for five minutes and filtered. The solvent was then removed by using a rotary evaporator at 40°C. The samples were left at -25°C.

2.1.1.1. Analysis for fatty acids

To obtain the fatty acid methyl esters, 2% sulphuric acid (v/v) in methanol was used (Christie, 1990). N-hexane was added to the fatty acid methyl esters and isolated by gas chromatography and flame-ionization detection (Shimadzu GC 17 Ver.3) coupled with a Glass GC 10 software. Nitrogen was used as a carrier gas with a flow ratio of 0.8 ml/min. and a capillary column of 25 m in length and 0.25 mm in diameter; Permabound 25 (Macherey-Nagel, Germany) was used to conduct the chromatographic separation.

2.1.1.2. Chromatographic analysis and quantification of lipid-soluble vitamins and sterols.

Lipid-soluble vitamins and phytosterols were obtained from the lipid fraction based on the method of Sánchez-Machado et al. (2002). The samples were suspended in acetonitrile/methanol (75/25 v/v), and 50 mL was added to the HPLC (Shimadzu, Japan). A Supelcosil TM LC18 (250 x 4.6 mm, 5 mm, Sigma, USA) was used as column and acetonitrile/methanol (75/25, v/v) for the mobile phase. The temperature of the column was kept at 40°C. The wavelength was 320 nm for retinol (vitamin A) and retinol acetate; 215 nm for d-tocopherol, vitamin D, a-tocopherol, and a-tocopherol acetate; 202 nm for phytosterols; and 265 nm for vitamin K1 (López-Cervantes et al., 2006). The results of the analyses were measured as μg/g.

2.2. Extraction of plant material for phenolics

Five ml 80% methanol was used to homogenize the flavonoid and phenolic acids. The extracts were centrifuged at 5000 rpm at +4°C, and dimethyl sulfoxide (DMSO) was used to provide a reserve solution (Kursat et al., 2011).

Table 1. The localities of *Tanacetum densum* subsp. *amani* and *Tanacetum densum* subsp. *laxum*.

Taxa	Locality	Coordinates	Herbarium number
Tanacetum densum (Lab.) Schultz Bip subsp. *laxum*	Elazig, Baskil, Bolucuk village, Hasan mountain, 1600 m.	38°.59′36402”N, 38°.86 ′46655”E	FUH 10101
Tanacetum densum (Lab.) Schultz Bip subsp. *amani* Heywood	Elazig, Baskil, Bolucuk village, Hasan mountain, 1800-1900 m.	38°.59′45642“N, 38°.86′49200”E	FUH10102

2.2.1. Determination of total phenolics

Total phenolics were evaluated using the Folin–Ciocalteu method (Singleton et al., 1999). 100 µl of the extracts was added to the mixture that included 200 µl of Folin– Ciocalteu reagent and 3.16 ml of H_2O. The samples were kept at room temperature for three min. The extracts were left at room temperature for two hours after anhydrous sodium carbonate (20%; w/v) was added to the mixture. The absorbance of the samples was analyzed at 765 nm (Sarhan et al., 2013).

2.2.2. Chromatographic conditions of the flavonoids

Chromatographic analysis was performed by Zu et al. (2006). The column was a PREVAIL C18 reversed-phase column (15x4.6mm, 5µm, USA), and the mobile phase was methanol/water/acetonitrile (46/46/8, v/v/v) including 1.0% acetic acid (Zu et al., 2006). The chromatographic peaks were confirmed by determining retention times with those of the standards. Resveratrol, quercetin, naringenin, naringin, catechin, myricetin, morin, rutin, kaempferol and vanillic acid, cinnamic acid, caffeic acid, and rosmarinic acid were analyzed by DAD following RP-HPLC. The flow ratio was 1.0 ml/min, and the chromatographic studies were performed at 25°C.

2.3. Antioxidant activity

2.3.1. DPPH radical scavenging capacity

The DPPH radical was prepared afresh based on the method by Liyana-Pathirana & Shahidi (2005). A 4. 0 ml DPPH solution was added to 25, 50, 100, 150, and 250 µL of the extract. The complex was kept in the dark for 30 minutes. The absorbance at a wavelength of 517 nm was measured spectrophotometrically. 1 µM quercetin was used as a reference (Liyana-Pathirana & Shahidi, 2005). The results were measured by using the following formula: DPPH radical scavenging activity (%)=[(Abs_control– Abs_sample)]/(Abs control)] x 100. The abs control is the absorbance of DPPH radical + methanol; the Abs_sample is the absorbance of DPPH radical + sample extract/standard.

2.3.2. ABTS 2.2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt assay

The ABTS radical cation assay was measured based on the method of Re et al. (1999). To obtain ABTS radical cation, the ABTS radical cation ($ABTS^{\bullet+}$) and 7 mM ABTS were added to 2.45 mM potassium persulfate. The solution was kept for 12–16 h at room temperature. The ($ABTS^{\bullet+}$) solution was dissolved in water to measure an absorbance of 0.700 ± 0.020 at 734 nm. 3 ml ABTS solution was added to 25, 50, 100, 150, and 250 µL of samples, and absorption was measured over six min. Absorbance of the control (3.0 mL ($ABTS^{\bullet+}$) solution with 30 L water) was written as A_control. (Skotti et al., 2014). The ABTS radical cation scavenging capacity (%) equals [(Abs_control – Abs_sample)]/ (Abs_control)] x 100.

2.3.3. Ferric-reducing antioxidant power assay (FRAP)

The FRAP method was performed using the procedure of Benzie & Strain (1996). A sodium acetate buffer (300 mmol/l), a TPTZ solution in 40 mmol/l, and 20 mmol/l $FeCl_3$ (10:1:1; v/v) were used to prepare the FRAP reagent. The absorbance was measured at 593 nm after 10 min. The $FeSO_4$ solution (100-2000 mmol/L) was used to form the standard curve. The results were evaluated as mM Fe (II)/g.

2.4. Statistical analysis

All analyses were done using the SPSS 21.0 statistical program. The simple linear regression model was used to determine the correlation between antioxidant capacity (ABTS and DPPH) and total phenolic contents. Data taken from the present study was represented as mean values ± standard deviation.

3. Results and Discussion

Tanacetum L. have been used since ancient times as medicinal plants, but to understand their curative effect, comprehensive phytochemical and pharmacological studies are required (Ivanescu et al., 2018). It was found that *T. densum* subsp. *amani* (55.84±1.31%) had a higher saturated fatty acid content than *T. densum* subsp. *laxum* (44.13±1.28%). Palmitic acid (C16:0) was the major saturated fatty acid while linoleic acid (C18:2 n6) was the major unsaturated fatty acid in two endemic subspecies (Table 2). Also, stearic acid (C18:0) was the second highest saturated fatty acid, and arachidic acid (C20:0) was the third highest saturated fatty acid in *T. densum* subsp. *laxum*. In addition, arachidic acid and stearic acid were found to be the saturated fatty acids in *T. densum* subsp. *amani.* The other saturated fatty acids in *T. densum* subsp. *laxum* were capric acid (C10:0) and myristic acid (C14:0). Demirpolat et al. (2019) determined that palmitic acid (C16:0) and stearic acid (C18:0) were the major saturated fatty acids in *T. densum* subsp. *eginense.* On the other hand, Rezaei et al. (2017) determined that *T. parthenium* had mainly palmitic acid (57.27%) and myristic acid (14.7%), saturated fatty acids. Oleic acid (C18:1 n9), linoleic acid (18:2 n6c), and a-linolenic acid (C18:3 n3) were the unsaturated fatty acids in the present study (Table 2). It was shown that the total unsaturated fatty acid compositions of *T. densum* subsp. *laxum* was 60.1±1.61% whilst the total unsaturated fatty acid compositions of *T. densum* subsp. subsp. *amani* was 44.13±1.28%. The health effect of dietary fats from the plants are based on the polyunsaturated and monounsaturated fatty acid composition (Ayaz et al., 2017). In addition, the ω3 (alpha linolenic acid 18:2) and the ω6 (cis linoleic acid; 18:2) fatty acids are essential fatty acids (called vitamin F), and they cannot be synthesized by humans and must be taken in food (Konukoglu, 2008; Kam&Saydan Kanberoglu, 2019). This study showed that *T. densum* subsp. *laxum* has a higher linoleic acid (C18:2 n6; 28.31±2.61%) and

Table 2. Fatty acid compositions % of *Tanacetum* taxa.

Fatty Acids (%)	*Tanacetum densum* subsp. *laxum*	*Tancetum densum* subsp. *amani*
10:0	2.09±0.54	-
14:0	2.55±0.78	-
16:0	24.54±1.37	37.49±2.34
18:0	5.72±0.97	9.47±0.89
20:0	4.94±0.85	8.88±1.1
ΣSaturated fatty acids	39.84±1.37	55.84±1.31
18:1 n9	11.75±1.13	11.37±1.01
18:2 n6c	28.31±2.61	17.02± 1.58
18:3n3	16.22±1.1	10.59±1.26
20:4n6	3.83±0.76	5.15±0.93
ΣUnsaturated fatty acids	60.11±1.61	44.13±1.28

Table 3. Lipid soluble vitamin and sterol contents of *Tanacetum* taxa (µg/g).

Lipid-soluble vitamins (µg/g)	*Tanacetum densum* subsp. *laxum*	*Tancetum densum* subsp. *amani*
K1	1.5±0.22	0.75±0.19
K2	-	-
R-tocopherol	0.55±0.1	0.8±0.14
D2	0.05±0.01	0.05±0.01
D3	0.1±0.01	0.2±0.01
a-tocopherol	7.3±0.67	5±0.57
Retinol	-	-
Retino acetate	0.6± 0.1	0.65±0.26
Ergosterol	0.75±0.1	9.35±1.12
Stigmasterol	59.45±3.11	63.55±3.21
B-sitosterol	0.7±0.1	0.05±0.01

α-linolenic acid (C18:3 n3; 16.22±1.1%) content than the linoleic acid (C18:2 n6; 17.02±1.58%) and α-linolenic acid (C18:3 n3; 10.59±1.26%) content of *T. densum* subsp. *amani.* However, Demirpolat et al. (2019) indicated that linolenic acid (C18:3 n3) was a major unsaturated fatty acid in *T. densum* subsp. *eginense.* Oleic acid (C18:1 n9) was identified as a monounsaturated fatty acid the contents which of were similar in this study (Table 2). The differences in the major fatty acids in the *T. densum* taxa may be due to environmental conditions because the concentrations and compositions of oils are affected by environmental conditions (Seiler, 1994). Also, it has been reported that fatty acid composition is affected by the latitude of the growing field, and it was found that there is a negative correlation between latitude and linoleic acid (Turhan et al., 2010). Current results showed that there is a negative correlation between the linoleic acid (C18:2 n6) content and the latitude of two endemic *Tanacetum* taxa. Yur et al. (2017) indicated that *T. haussknechtii* (Bornm.) Grierson had palmitic acid (C16:0), linoleic acid (C18:2 n6), and linolenic acid (C18:3 n3) as major fatty acids while Eyol et al. (2017) found that *Tanacetum zahlbruckneri* had palmitic acid as the major saturated fatty acid (21.28%-33.78%), and α-linoleic acid (15.3% and 18.09%) and α-linolenic acid (17.17%) were major unsaturated fatty acids. Besides, the fatty acid composition (especially of linoleic acid and linolenic acids) are significant biochemical tools for solving the taxonomical problems in the different systematic levels (Ayaz et al., 2017). This study demonstrated that the ω6/ω3 ratio of *T. densum* subsp. *laxum* (1.74) and *T. densum* subsp. *amani* (1.60) was found to be similar.

The present study demonstrated that *T. densum* subsp. *laxum* and *T. densum* subsp. *amani* had the lowest lipid-soluble vitamins and ergosterol and ß-sitosterol content (Table 3). However, the stigmasterol content of the two *Tanacetum* taxa studied was found to be between 59.45±3.11 µg/mg and 63.55±3.21 µg/mg. Plant sterols are important elements that include the interaction between the free hydroxyl group protein and phospholipids and they protect the cells against cancer and cardiovascular diseases (Tosun et al., 2018; Beyzi et al., 2019). Furthermore, stigmasterol is an unsaturated plant sterol found in several medicinal plants and plays a significant role in the biosynthesis of androgens, estrogens, vitamin D3, and corticoids (Kaur et al., 2011). Azizudin & Choudhary (2008) showed that β-sitosterol and stigmasterol were found in *T. polycephalum.* In another study, Ivanescu et al. (2018) showed that all three *Tanacetum* species (*T. vulgare, T. macrophyllum,* and *T. corymbosum*) contain beta-sitosterol, stigmasterol and campesterol, and traces of ergosterol. They found a high β-sitosterol content (530.78 µg/g dw -696.32 µg/g dw) compared to the present study (Ivanescu et al., 2018).

Chandler et al. (1982) also found β -sitosterol to be the major sterol in *Tanacetum*. This research may be the first report regarding the lipid-soluble vitamin content of the two endemic *Tanacetum* taxa.

The studies demonstrated that phenolic content is responsible for the antioxidant activities of plants (Wojdylo et al., 2007; Arituluk et al., 2016). Phenolic content plays a preventive role against diabetes, cancer, cardiovascular disease, and Alzheimer's disease as well as in inducing DNA, cell adhesion, cell proliferation, and blocking signaling pathways (Huang et al., 2010; Gutierrez-Grijalva et al., 2016). Because synthetic antioxidants have harmful effects on health, there is a growing interest in natural antioxidants (Ahmed et al., 2011). This study found that the total phenolic contents of *T. densum* subsp. *laxum* and *T. densum* subsp. *amani* was 188.94±3.24 µg/mg and 137.01±2.47 µg/mg, respectively (Table 4). Tepe&Sokmen (2007) found that the total phenolic content of *T. densum* subsp. *amani* was 158.44 ± 2.17 µg/mg. However, Caniklioglu et al. (2018) determined that the total phenolic amount was 84.94±0.009 mg/g in the ethanol extracts of *T. densum* subsp. *eginense*. Also, catechin amounts were found at high levels in the present study (1,299.4±7.52 µg/mg-5,796.9±8.12 µg/mg). Though Michel et al. (2020) indicated that kaempherol glycosides are the main phenolic in the Asteracea, but present study showed that the major phenolic compound of *T. densum* taxa is catechin. Similarly, Gecibesler et al. (2016) found that catechin was a principal component of *Tanacetum.* The other phenolics studied were in low amounts or absent (Table 4). Zengin et al. (2019) indicated in their study that *Tanacetum* had various phenolics, but most of them were small amounts. On the other hand, the current study determined that *T. densum* subsp. *laxum* and *T. densum* subsp. *amani* had the highest vanillic acid (1,543.7±6.12 µg/mg and 1070.3±4.91 µg/mg, respectively) and caffeic acid (1,234.9±5.64 µg/mg and 790±3.89 µg/mg, respectively) contents (Table 5). Cinnamic acid was found trace amounts in the present study. It was also found that the rosmarinic acid amounts in *T. densum* taxa was the lowest (Table 5). Muresan et al. (2015) showed that *Tanacetum* had caffeic acid, ferulic acids, chlorogenic acid, rutin and quercetin, and kaempferol. They also showed that *Tanacetum* had good antioxidant activity (Muresan et al., 2015). Esmaeili et al. (2010) determined that the six *Tanacetum* species except for *T. densum* exhibited antioxidant activity. They also found that caffeic acid, ferulic acid, luteolin, apigenin, and rutin were major phenolic compounds in the *Tanacetum* species (Esmaeili et al., 2010). The differences in the phenolic amounts may originate from the growing conditions of the taxa because environmental factors including climate, weather, and sunlight exposure effect the phenolic content (Bahukhandi et al., 2013). Hashim et al. (2020) also indicated that ecological conditions affect antioxidant activity and the number of dominant constituents in endemic medical plants. In addition, it was reported that collection time has an important effect on the phenolic content (Varga et al., 2016). Climatic conditions, specifically the altitude of growing area, also effect the quantitative content of secondary metabolites such as phenolics at flowering time (Spitaler et al., 2008). The present results may suggest that the quantitative differences in the total phenolics, quercetin, catechin, caffeic acid, rosmarinic acid, and vanillic acid content of two endemic *T. densum* taxa exist because of the altitude. On the other hand, it has been suggested that qualitative and quantitative differences in the flavonoid and total phenolics could be used as taxonomical markers for tribes and subtribes of Asteraceae (Emerenciano et al., 2001; Sytar et al., 2018). In this study, while the phenolic content is similar in the two taxa studied, the phenolic amounts are different. The present study demonstrated that the two studied endemic taxa of *T. densum* had high DPPH (except for 25 µl) and ABTS radical scavenging activities (Tables 6, 7). The present DPPH results agreed with the study by Yur et al. (2017) who indicated that the methanol extracts from *Tanacetum* represented the highest DPPH activity. Also, Zengin et al. (2019) showed that the water and methanol extracts from *Tanacetum* had high phenolic content and high ABTS and DPPH radical scavenging capacities. However, Caniklioglu et al. (2018) found that the DPPH (20.64±0.26%-48.13±1.37%) and the ABTS radical scavenging activities (12.65±0.23% and

Table 4. The results of total phenolics and flavonoid contents of *Tanacetum* taxa (µg/mg).

Taxa	Total phenolics	Rutin	Myricetin	Quercetin	Kaempherol	Catechin	Naringin	Naringenin	Resveratrol
Tanacetum densum subsp. *laxum*	188.94±3.24	3.8±0.27	0.7±0.01	93.5±2.15	18.7±0.94	1,299.4±7.52	-	8.5±0.64	8.1±0.97
Tancetum densum subsp. *amani*	137.01±2.47	2.1±0.12	-	33.21±.13	12.6±0.87	5,796.9±8.12	-	6.3±0.81	5.2±0.43

Table 5. The results of phenolic acid contents of *Tanacetum* taxa (µg/mg).

Taxa	Vanillic acid	Cinnamic acid	Caffeic acid	Rosmarinic acid
Tanacetum densum subsp. *laxum*	1,543.7±6.12	0.4±0.01	1,234.9±5.64	19.3±0.94
Tancetum densum subsp. *amani*	1070.3±4.91	0.2±0.01	790±3.89	2.3±0.4

Table 6. The DPPH % results of *Tanacetum* taxa

Taxa	25 µl	50 µl	100 µl	150 µl	250 µl	500 µl
Tanacetum densum subsp. *laxum*	16.07±1.11	87.5±2.41	89.28±2.34	92.64±2.2	94.8±1.96	93.66±2.12
Tancetum densum subsp. *amani*	16.94±0.97	47.14±1.37	68.83±2.71	93.83±2.25	94.9±1.74	94.64±2.36

Table 7. The ABTS % results and FRAP activities (mM Fe (II)/g) of *Tanacetum* taxa.

Taxa	25 µl	50 µl	100 µl	150 µl	250 µl	500 µl	FRAP
Tanacetum densum subsp. *laxum*	76.68±2.14	90.29±1.32	83.76±2.72	93.71±2.54	98.6±1.76	98.9±1.89	566.97±4.57
Tancetum densum subsp. *amani*	54.92±1.58	54.01±1.12	75.39±2.34	95.46±2.17	98.9±2.11	98.95±1.63	74.94±1.45

23.58±0.49%) in the ethanol extracts from *T. densum* subsp. *eginense* were lower than the present results. This study demonstrated that there is a strong correlation between total phenolics and ABTS (r^2:.716) and a moderate correlation between total phenolics and DPPH (r^2:.274). Furthermore, this study showed that *T. densum* subsp. *laxum* had higher FRAP activity (566.97±4.57 mM Fe (II)/g) than *T. densum* subsp. *amani* (74.94±1.45 M Fe (II)/g, Table 7). Studies in the literature showed that *Tanacetum* had potent reducing power activity (Savci et al., 2019; Zengin et al., 2019).

4. Conclusion

This study found that palmitic acid (C16:0) and stearic acid (C18:0) were the major saturated fatty acids and oleic acid (C18:1 n9), linoleic acid (18:2 n6), and a-linolenic acid (C18:3 n3) were the dominant unsaturated fatty acids. Also, the present study showed that the polyunsaturated fatty acid composition of *T. densum* subsp. *laxum* was higher (48.76±1.49%) than the poly unsaturated fatty acid composition (32.76±1.25%) of *T. densum* subsp. *amani*, but the mono unsaturated fatty acid compositions of *T. densum* taxa was similar. Besides, the ω6/ω3 of *T. densum* subsp. *laxum* (1.74) and *T. densum* subsp. *amani* (1.60) was determined to be at a similar ratio. However, this study also determined that the lipid soluble vitamin and sterol content of two endemic *Tanacetum* taxa were low except for the stigmasterol contents of the studied *Tanacetum* taxa. Current study demonstrated that catechin is main phenolic compound in the two endemic *T. densum* taxa. It was shown in the present study that *T. densum* subsp. *laxum* and *T. densum* subsp. *amani* had the highest catechin, vanillic acid and caffeic acid contents but the phenolic amounts, particularly catechin and quercetin, were different in the *T. densum* taxa. This study proposed that altitude may affect the biochemical contents of two endemic *Tanacetum densum* taxa. Moreover, this study determined that two endemic *Tanacetum* taxa had strong DPPH and ABTS radical scavenging capacities, and *T. densum* subsp. *laxum*, in particular, had the highest FRAP activity. This study found that there was a correlation between total phenolics and antioxidant capacity.

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