FABAD J. Pharm. Sci., 41, 17-25, 2016

RESEARCH ARTICLE

Antioxidant Activity, Total Phenolic and Flavonoid Contents of Some *Tanacetum* L. (Asteraceae) Taxa Growing in Turkey

Zekiye Ceren ARITULUK*°, İffet İrem TATLI ÇANKAYA*,
Ayşe Mine GENÇLER ÖZKAN**

Antioxidant Activity, Total Phenolic and Flavonoid Contents of Some Tanacetum L. (Asteraceae) Taxa Growing in Turkey

SUMMARY

In this study, five Tanacetum taxa (T. armenum (DC.) Schultz Bip., T. cadmeum (Boiss.) Heywood ssp. cadmeum, T. cilicicum (Boiss.) Grierson, T. praeteritum (Horw.) Heywood ssp. massicyticum Heywood, T. praeteritum (Horw.) Heywood ssp. praeteritum) collected from Antalya were investigated for their possible in vitro antioxidant activity. For this purpose, methanol extracts of the aerial parts of these plants were tested with four complementary methods, namely 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity, 2,2'-azinobis (3-ethylbenzothiazolin-6-sulphonic acid) (ABTS) radical cation scavenging capacity, cupric ion reducing antioxidant capacity (CUPRAC) and ferric-reducing antioxidant power (FRAP) assays. Moreover, total phenolic contents of plant extracts were determined by Folin-Ciocalteu's reagent and their flavonoid contents were determined by aluminum chloride colorimetric method. Results of present study revealed that, all plant extracts exhibited antioxidant effect with various potencies. Also it was determined that, T. praeteritum ssp. massicyticum, which showed the highest scavenging activity against DPPH radical (IC_{50}=197.82 µg/mL) and ABTS radical cation (191.41 mg Trolox equivalents/g extract) as well as possessed the highest cupric ion reducing antioxidant capacity (138.86 mg gallic acid equivalents/g extract) among the plants studied, had also the highest total phenolic (149.93 mg gallic acid equivalents/g extract) and flavonoid (33.42 mg quercetin equivalents/g extract) contents. Furthermore, T. cilicicum which showed lower activity than other plant extracts on DPPH, ABTS and CUPRAC assays, was determined to possess the highest ferric reducing antioxidant power (198.19 mg quercetin equivalents/g extract).

Key Words: *Tanacetum, Asteraceae, Antioxidant activity, Total phenolic content, Total flavonoid content, Antalya.*

Türkiye'de Yetişen Bazı Tanacetum L. (Asteraceae) Taksonlarının Antioksidan Aktivitesi, Total Fenolik ve Flavonoit İçerikleri

ÖZET

Bu çalışmada, Antalya ilinden toplanan beş Tanacetum taksonunun (T. armenum (DC.) Schultz Bip., T. cadmeum (Boiss.) Heywood ssp. cadmeum, T. cilicicum (Boiss.) Grierson, T. praeteritum (Horw.) Heywood ssp. massicyticum Heywood, T. praeteritum (Horw.) Heywood ssp. praeteritum) olası in vitro antioksidan aktivitesi araştırılmıştır. Bu amaçla, bitkilerin toprak üstü kısımlarından hazırlanan metanol ekstreleri, 2,2-difenil-1-pikrilhidrazil (DPPH) radikal süpürücü kapasite tayini, 2,2'-azinobis (3-etilbenzotiyazolin-6-sülfonik asit) (ABTS) radikal katyonu süpürücü aktivite tayini, bakır iyonu indirgeyici antioksidan kapasite (CUPRAC) tayini ve demir iyonu indirgeyici antioksidan güç (FRAP) tayini olmak üzere birbirini tamamlayıcı dört farklı yöntem ile test edilmiştir. Ayrıca, ekstrelerin içeriğindeki total fenolik madde miktarları Folin Ciocalteu reaktifi kullanılarak, total flavonoit miktarları ise alüminyum klorür kolorimetrik yöntemiyle tespit edilmiştir. Çalışmamızın sonuçları, test edilen tüm Tanacetum taksonlarının toprak üstü kısımlarından hazırlanan metanol ekstrelerinin değişik oranlarda antioksidan aktiviteye sahip olduğunu ortaya çıkarmıştır. Bu bitkiler arasında, en güçlü DPPH radikali (IC_{50}=197.82 µg/mL) ve ABTS radikal katyonu (191.41 mg troloks eşdeğer/g ekstre) süpürücü aktivite yanında, en yüksek bakır iyonu indirgeyici antioksidan kapasiteye sahip olan (138.86 mg gallik asit eşdeğer/g ekstre) T. praeteritum ssp. massicyticum'un, en yüksek total fenolik madde ve flavonoit içeriğine de sahip olduğu (sırasıyla 149.93 mg gallik asit eşdeğer/g ekstre ve 33.42 mg kersetin eşdeğer/g ekstre) tespit edilmiştir. Bunun yanında, DPPH, ABTS ve CUPRAC testlerinde, diğer ekstrelere göre daha düşük aktivite gösteren T. cilicicum'un, en yüksek demir iyonu indirgeyici antioksidan güce sahip olduğu (198.19 mg kersetin eşdeğer/g ekstre) belirlenmiştir.

Anahtar kelimeler: *Tanacetum, Asteraceae, Antioksidan aktivite, Total fenolik içeriği, Total flavonoit içeriği, Antalya.*

Received: 13.02.2017
Revised: 06.03.2017
Accepted: 06.03.2017

* Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Botany, 06100 Sıhhiye, Ankara, TURKEY.
** Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, 06100 Tandoğan, Ankara, TURKEY.

° Corresponding Author:
Tel: +903123051089,
Fax: +903123114777,
E-mail: zceren@hacettepe.edu.tr

INTRODUCTION

Human body has a complex defense system composed of natural enzymatic and non-enzymatic antioxidants against the harmful effects of free radicals and other oxidants. It has been reported by many researchers that there is an inverse correlation between the occurence of the essential antioxidants in plasma and subsequent risk to develop degenerative disorders such as cardiovascular, neurodegenerative and inflammatory diseases, cancers and Alzheimer's disease (Alam et al., 2013; Siti et al., 2015; Wojtunik-Kulesza et al., 2016). Antioxidants -many belonging to the phenol family- present abundantly in natural plant sources and our nutrients are considered as the solution in preventing many common health disorders result from oxidative stress (Krishnaiah et al., 2011). Accordingly over the past years, natural antioxidants have become of increasing interest because of their possible beneficial biological properties in this sense (Ivanova et al., 2005; Kähkönen et al., 1999; Miser-Salihoğlu et al., 2010; Şenol et al., 2010; Tawaha et al., 2007; Wojdyło et al., 2007).

The genus *Tanacetum* L., as the third largest genus of the Asteraceae family, is composed of nearly 160 species found in Mediterranean region, central and eastern Asia, and some parts of northern America (Sonboli et al., 2012). In Turkey, *Tanacetum* genus is represented by 47 species or 61 taxa including subspecies and varieties and 27 of those are endemic (Güner et al., 2012). Several species of *Tanacetum* genus have been used traditionally as herbal remedies in a variety of health conditions including pain, fever, inflammation, arthritis, migraine, respiratory and gastrointestinal disorders. The members of this genus are rich in essential oils, sesquiterpenes and phenolic compounds which are responsible for their biological activities such as antiinflammatory, antimicrobial, antifeedant, cytotoxic, insecticidal etc. (Abad et al., 1995; Gören et al., 2002). *T. parthenium* (L.) Schultz Bip which is known as feverfew is the most prominent species with its efficacy in prevention of migraine and according to the chemical market reporters amongst the 50-top selling supplements in USA (Minkwitz, 1999). Many researchers have stated that parthenolide was the major bioactive component. Wu et al.(2006) have revealed for the first time that alcoholic extract of feverfew also possesses strong free radical scavenging activity resulting from the the presence of some lipophilic flavonoids. At the end, they reached to the assumption that consumption of *T. parthenium* extract might be able to provide some further health benefits against oxidative stress, except its benefits for treating migraine (Wu et al., 2006). This pioneering result made way for many reports concerning antioxidant activity of extracts and essential oils of a large number of members of this genus (Baranauskienė et al., 2014; Esmaeili et al., 2010; Esmaeili and Amiri, 2011; Polatoglu et al., 2012; Tepe and Sokmen, 2007; Wu et al., 2006).

Hence various antioxidants provide different defence mechanisms against the effects of excessive oxidations, it is very difficult to measure the antioxidant activity directly. For many methods commonly used to assess the antioxidant activity, the frequent lack of an authenticated substrate in the procedure is the main limitation. Moreover, when it comes to the plant extracts with a huge chemical complexity of natural antioxidants, numerous compounds with different functional groups, polarity and chemical features, there are many variables influencing the results. Besides, *in vitro* assays can only rank antioxidant activity according to their specific reaction system with a limited relevance to *in vivo* health protective mechanisms. Taking into account all of these, for the sake of avoiding possible scattered results it's an obligation to use multiple-assay approach in screening of antioxidant activity of the extracts. Therefore in this study, the *in vitro* antioxidant activity of methanol extracts of the aerial parts of *T. armenum, T. cadmeum* ssp. *cadmeum*, *T. cilicicum*, *T. praeteritum* ssp. *massicyticum*, *T. praeteritum* ssp. *praeteritum* were tested with four complementary methods namely DPPH radical scavenging capacity, ABTS radical cation scavenging capacity, cupric ion reducing antioxidant capacity (CUPRAC) and ferric-reducing antioxidant power (FRAP) assays (Antolovich et al., 2002; Badarinath et al, 2010; Sacchetti et al., 2005). To our knowledge, there is no detailed information available for the antioxidant activity of those *Tanacetum* taxa growing in Antalya.

MATERIALS AND METHODS

Plant materials

All investigated plant materials were collected at their flowering period from Antalya, Turkey and identified by authors using Flora of Turkey (Grierson, 1975). Voucher specimens have been deposited in the Herbarium of Hacettepe University, Faculty of Pharmacy, Ankara, Turkey (HUEF) under related HUEF codes. Collection details and herbarium codes of plants are given in Table 1.

Table 1. Collection details and herbarium numbers of the plants

Plant taxa	Collection sites	Collection dates	Altitudes	HUEF codes
T. armenum (DC.) Schultz Bip.	Gündoğmuş	24.06.2014	2060 m	14058
[a]*T. cadmeum* (Boiss.) Heywood ssp. *cadmeum*	Gazipaşa	09.07.2013	2005 m	13023
T. cilicicum (Boiss.) Grierson	Manavgat	26.06.2014	1093 m	14064
[a]*T. praeteritum* (Horw.) Heywood ssp. *massicyticum*	Kaş, Gömbe	07.07.2013	1864 m	13022
[a]*T. praeteritum* (Horw.) Heywood ssp. *praeteritum*	Kaş, Gömbe	07.07.2013	1882 m	13021

[a]Endemic taxa

Chemicals

Gallic acid was purchased from Merck. 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzothiazolin-6-sulphonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,9-dimethyl-1,10-phenanthroline (neocuproine), Folin-Ciocalteu's phenol reagent, quercetin and other chemicals were purchased from Sigma.

Preparation of the extracts

Air-dried and powdered aerial parts of the plant materials (20 g) were extracted with methanol (3 x 500 mL) in a water-bath at 60 °C, concentrated to dryness under reduced pressure and lyophilized in vacuo.

DPPH radical scavenging capacity assay

DPPH radical scavenging capacity of each extract was determined by the method of Brand-Williams et al. (1995) with slight modification. 1mM DPPH radical solution was prepared in ethanol. 50 µL of this solution was mixed with 150 µL of different concentrations of the extract (200 µg/mL to 12.5 µg/mL) and the reference (200 µg/mL to 1.25 µg/mL) dissolved in ethanol. The reaction mixture was incubated for 30 minutes in the dark, then absorbance was measured at 517 nm. Quercetin was used as reference. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula:

Inhibition % = $[(A_{blank}-A_{sample})/A_{blank}]\times100$, where A_{blank} is the absorbance of the blank (containing ethanol instead of sample) and A_{sample} is the absorbance of the extracts or reference. The assay was carried out in triplicate and the results were expressed as average values with SEM (standard error mean). The half-maximal inhibitory concentration (IC_{50}) value for each extract was calculated from the plotted graph of scavenging activity against the concentrations of the sample.

ABTS radical cation scavenging activity assay

ABTS radical cation scavenging activity assay was carried out according to the method described by Re et al. (1999) with slight modification. ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation ($ABTS^{\cdot+}$) was generated by reacting ABTS stock solution with 2.45 mM potassium persulfate ($K_2S_2O_8$) and allowing the mixture to stand in the dark at room temperature for 12-16 hours. $ABTS^{\cdot+}$ solution was diluted with ethanol to an absorbance of 0.700 ± 0.02 nm at 734 nm before use. 200 µL of this solution was mixed with 20 µL of the extract and different concentrations of reference (400 µg/mL to 12.5 µg/mL) dissolved in ethanol. The reaction mixture was incubated for 6 minutes at room temperature in the dark, then absorbance was measured at 734 nm. Trolox was used as reference. The assay was carried out in triplicate. Results were expressed in terms of Trolox equivalent antioxidant capacity (TEAC) using a calibration curve.

Cupric ion reducing antioxidant capacity (CUPRAC) assay

Cupric ion reducing antioxidant capacity assay was carried out according to the method described by Apak et al. (2004) with slight modification. Briefly, 50 µL of copper(II) chloride ($CuCl_2$) solution ($1.0x10^{-2}$ M), 50 µL of neocuproine solution ($7.5x10^{-3}$ M), 50 µL of ammonium acetate (NH_4Ac) buffer solution at pH 7.0 (1.0 M) were added to each well in 96 well plate and mixed. Then 25 µL of extracts or different concentrations of reference (800 µg/mL to 25 µg/mL) and 25 µL of distilled water were added to the initial mixture, separately. The absorbance of the final solution was measured at 450 nm after 30 minutes standing at room temperature in the dark. Increased absorbance of the reaction mixture indicates increased reduction capability. The assay was carried out in triplicate. Gallic acid was used as reference. Results were presented as gallic acid equivalents (mg/g extract) using a calibration curve.

Ferric reducing antioxidant power (FRAP) assay

Reducing power ability of each extract was determined by the method of Oyaizu (1986). Briefly, extracts and different concentrations of reference (160 µg/mL to 2.5 µg/mL) were dissolved in ethanol. 20 µL of these solutions were mixed with 50 µL of 0,2 M sodium phosphate buffer (pH 6.6) and 1% (w/v) potassium ferricyanide [$K_3Fe(CN)_6$]. This mixture was incubated at 50°C for 20 minutes. After incubation, the reaction mixture was acidified with 50 µL of 10% (w/v) trichloroacetic acid. After the

mixture was shaken vigorously, 50 µL of distilled water and 10 µL of 0.1% (w/v) iron(III) chloride ($FeCl_3$) were added to the 50 µL of this mixture. The absorbance was measured at 700 nm after 30 minutes of incubation. Increased absorbance of the reaction mixture indicated increased reducing power. The assay was carried out in triplicate. Quercetin was used as reference. Results were presented as quercetin equivalents (mg/g extract) using a calibration curve.

Determination of total phenolic contents

Phenolic contents of plant extracts were determined using Folin-Ciocalteau's reagent according to the method described by Slinkard and Singleton (1977) with slight modification. Folin-Ciocalteau's reagent was diluted with distilled water (1:10). 100 µL of this solution was mixed with 20 µL of extract and different concentrations of reference (400 µg/mL to 25 µg/mL) dissolved in ethanol. After the mixture was shaken, 80 µL of sodium carbonate (Na_2CO_3) solution (7.5%) was added. The reaction mixture was kept at room temperature for 2 hours in the dark, then absorbance was measured at 765 nm. The assay was carried out in triplicate. Gallic acid was used as reference. The total phenolic contents of the extracts were expressed as gallic acid equivalents (mg/g extract) using a calibration curve.

Determination of total flavonoid contents

Flavonoid content of each extract was determined by the aluminum chloride colorimetric method described by Chang et al. (2002) with slight modification. 25 µL of extract and different concentrations of reference (800 µg/mL to 12.5 µg/mL) dissolved in ethanol were mixed with 75 µL of 95% ethanol, 5 µL of 10% aluminum chloride ($AlCl_3$), 5 µL of 1 M potassium acetate (KCH_3COO) and 140 µL of distilled water. After incubation at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415 nm. The assay was carried out in triplicate. Quercetin was used as reference. Results were presented as quercetin equivalents (mg/g extract) using a calibration curve.

RESULTS AND DISCUSSION

DPPH radical scavenging capacity

The DPPH radical scavenging assay is based on hydrogen atom donation of antioxidants which gives rise to the reduced form of DPPH radical (Alam et al., 2013). DPPH radical scavenging capacities of methanol extracts of *Tanacetum* taxa were tested at 25, 50, 100, 200 and 400 µg/mL concentrations. The inhibition percents of plant extracts on DPPH are summarized in Table 2. All plant extracts were found to possess concentration-dependent inhibitory activity against DPPH radical. IC_{50} values for DPPH radical scavenging capacity are given in Table 3. A lower IC_{50} value corresponds to a higher antioxidant activity of the plant extract. The highest scavenging activity on DPPH radical was shown by *T. praeteritum* ssp. *massicyticum* with 197.82 µg/mL of IC_{50} value and *T. praeteritum* ssp. *praeteritum* with 200.38 µg/mL of IC_{50} value. The lowest activity on DPPH has shown by *T. cilicicum* with 249.17 µg/mL of IC_{50} value. It was found that quercetin showed much higher activity on DPPH radical (IC_{50}=12.24 µg/mL) than plant extracts.

Table 2. The inhibitory effects of plant extracts on DPPH radical

	Percentage of inhibition ± SEM[a] against DPPH radical				
Plant extract	**25 µg/mL**	**50 µg/mL**	**100 µg/mL**	**200 µg/mL**	**400 µg/mL**
T. armenum	2.98±1.00	3.73±1.05	20.44±0.86	42.04±0.50	81.67±1.78
T. cadmeum ssp. *cadmeum*	5.33±0.90	13.23±2.19	21.91±0.99	43.53±2.41	82.80±1.70
T. cilicicum	6.91± 0.23	13.02±0.72	22.91±0.63	41.66±0.98	77.61±0.51
T. praeteritum ssp. *massicyticum*	9.61±0.82	17.63±0.96	34.69±1.49	63.08±1.21	83.10±0.21
T. praeteritum ssp. *praeteritum*	10.16±0.47	15.32±1.05	31.75±0.67	62.47±1.10	84.41±0.39

[a]SEM: Standard error mean

Table 3. DPPH radical scavenging capacity of plant extracts

Plant extract	**IC_{50} value** (µg/mL)
T. armenum	247.57
T. cadmeum ssp. *cadmeum*	236.58
T. cilicicum	249.17
T. praeteritum ssp. *massicyticum*	197.82
T. praeteritum ssp. *praeteritum*	200.38
Quercetin	12.24

ABTS radical cation scavenging activity

ABTS radical cation scavenging activity assay, which has been used to measure the total antioxidant activity of pure substances, body fluids and plant materials, is based on neutralization of the ABTS radical cation in the presence of antioxidants (Shahidi and Zhong, 2015). ABTS radical cation scavenging activity of the plant extracts was determined in accordance with the equation (y=1.2606x-0.5268, R^2=0.9946) of Trolox calibration curve. The results are expressed in terms of Trolox equivalent antioxidant capacity (TEAC) in Table 4. A higher TEAC value corresponds to a greater antioxidant activity of the plant extract. Similar to DPPH assay, the highest scavenging activity against ABTS radical cation was shown by *T. praeteritum* ssp. *massicyticum* (191.41 mg TE/g extract) and the lowest activity was shown by *T. cilicicum* (66.98 mg TE/g extract). Compared with DPPH asay, a higher level of antioxidant activity was also obtained from *T. cadmeum* ssp. *cadmeum* (167.96 mg TE/g extract). ABTS assay is applicable for both hydrophilic and lipophilic antioxidants (Abad et al., 1995). So, it can be suggested that near hydrophilic compounds, lipophilic compounds may be, in part, responsible for high antioxidant activity of *T. cadmeum* ssp. *cadmeum*.

Table 4. ABTS radical cation scavenging activity of plant extracts

Plant extract	TEAC[a] (mg TE/g extract)
T. armenum	71.16
T. cadmeum ssp. *cadmeum*	167.96
T. cilicicum	66.98
T. praeteritum ssp. *massicyticum*	191.41
T. praeteritum ssp. *praeteritum*	124.09

[a]TEAC: Trolox equivalent antioxidant capacity

Cupric ion reducing antioxidant capacity (CUPRAC)

Antioxidants can not only allow scavenging of free radicals by their electron donating capability, but also reduce higher valent elements such as copper, iron to their lower valence state. The redox potential of an antioxidant is an important indicator of its efficacy (Shahidi and Zhong, 2015). The CUPRAC assay is a redox potential-based method in which the copper(II)-neocuproine complex, as a chromogenic oxidant, is reduced to copper(I)-neocuproine chelate by antioxidants (Apak et al., 2004). Cupric ion reducing antioxidant capacities of the plant extracts were determined according to the equation (y=0.0093x+0.0188, R^2=0.9979) as gallic acid equivalent (mg/g extract). The results are given in Table 5. The highest activity was shown by *T. praeteritum* ssp. *massicyticum* (138.86 mg GAE/g extract) in the CUPRAC assay. *T. cadmeum* ssp. *cadmeum* (96.92 mg GAE/g extract) and *T. praeteritum* ssp. *praeteritum* (83.12 mg GAE/g extract) showed also high activity in this assay. The lowest activity was observed in *T. cilicicum* (39.21 mg GAE/g extract) and *T. armenum* (40.56 mg GAE/g extract), with with very close values to each other.

Table 5. Cupric ion reducing antioxidant capacity (CUPRAC) of plant extracts

Plant extract	Antioxidant capacity (mg GAE[a]/g extract)
T. armenum	40.56
T. cadmeum ssp. *cadmeum*	96.92
T. cilicicum	39.21
T. praeteritum ssp. *massicyticum*	138.86
T. praeteritum ssp. *praeteritum*	83.12

[a]GAE: Gallic acid equivalent

Ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power assay is also a redox potential-based method like CUPRAC which uses iron as the oxidant instead of copper. Ferric reducing antioxidant power of the plant extracts were determined according to the equation (y=0.0021x+0.0118, R^2=0.9922) as quercetin equivalent (mg/g extract). The results are given in Table 6. In contrast to the results of other assays, *T. cilicicum* (198.19 mg QE/g extract) possessed the highest activity in the FRAP assay. *T. praeteritum* ssp. *massicyticum* showed also very high activity (197.24 mg QE/g extract). The lowest activity was shown by *T. armenum* with 105.81 mg QE/g extract.

Table 6. Ferric reducing antioxidant power (FRAP) of plant extracts

Plant extract	Antioxidant capacity (mg QE[a]/g extract)
T. armenum	105.81
T. cadmeum ssp. *cadmeum*	162.32
T. cilicicum	198.19
T. praeteritum ssp. *massicyticum*	197.24
T. praeteritum ssp. *praeteritum*	146.92

[a]QE: Quercetin equivalent

Total phenolic and flavonoid contents

The results of many of the studies indicate that phenolic compounds are major contributors to antioxidant activity of plant extracts (Rice-Evans et al., 1997). For this reason, total phenolic and flavonoid contents are also evaluated in most of the studies on antioxidant activity of plant extracts. Total phenolic contents of plant extracts were estimated by the Folin-Ciocalteu's colorimetric method (Slinkard and Singleton, 1977) using gallic acid as a reference phenolic compound, while their total flavonoid contents were determined by the aluminum chloride colorimetric method (Chang et al., 2002) using quercetin as reference. Total phenolic contents were calculated according to the equation ($y=0.0056x+0.0466$, $R^2=0.9978$) obtained from calibration curve as gallic acid equivalent, while total flavonoid contents were calculated according to the equation ($y=0.004x+0.0514$, $R^2=0.9967$) obtained from calibration curve as quercetin equivalent (mg/g extract). As shown in Table 7, the amount of total phenolics in plant extracts varied from 33.14 to 149.93 mg GAE/g extracts and the total flavonoid contents varied from 7.69 to 33.42 mg QE/g extracts. The highest total phenolic and flavonoid levels have been detected in *T. praeteritum* ssp. *massicyticum* which showed highest activity in DPPH, ABTS and CUPRAC tests and very high activity in FRAP test.

Table 7. Total phenolic and flavonoid contents of plant extracts

Plant extract	Total phenolic content (mg GAE[a]/g extract)	Total flavonoid content (mg QE[b]/g extract)
T. armenum	58.86	7.69
T. cadmeum ssp. *cadmeum*	131.24	23.83
T. cilicicum	33.14	18.73
T. praeteritum ssp. *massicyticum*	149.93	33.42
T. praeteritum ssp. *praeteritum*	112.67	10.71

[a]GAE: Gallic acid equivalent

[b]QE: Quercetin equivalent

The members of *Tanacetum* genus have been used in traditional medicine for various ailments including pain, fever, inflammation, arthritis, migraine, respiratory and gastrointestinal disorders since ancient times. Among them *T. parthenium* (L.) Schultz Bip. and *T. vulgare* L. are more-prominent species in terms of extensive folk usage. For this reason, many researches have been conducted to characterize a variety of bioactivities they have including antioxidant activity. Wu and her colleagues reported that *T. parthenium* powder extracted by 80% alcohol contained camphor, parthenolide, luteoline in various amounts and have strong DPPH free radical scavenging activity of 84.4% and also moderate Fe^{2+}-chelating capacity of 53.1%. Authors indicated that antioxidant potency of the feverfew extract might result from the existence of luteolin, parthenolide and some other compounds they couldn't identify clearly from the HPLC-UV chromatographic profiles (Wu et al., 2006). In our research, we obtained the similar results regarded to the activity with much lower concentrations for each of the plant extracts. In another study, antioxidant activity of methanol extracts of flower, leaf and stem of *T. cilicicum* were investigated by various assays. The results of this study demonstrated that leaf, flower and stem of this species showed DPPH scavenging activity with IC_{50} values of 25.95 µg/mL, 30.26 µg/mL and 117.48 µg/mL, respectively (Gecibesler et al., 2016). In our study, methanol extract prepared by above ground parts of *T. cilicicum* showed a lower activity (IC_{50}=249.17 µg/mL).

In another study, besides their total phenolic contents, antioxidant activity of methanol extracts of *T. densum* (Lab.) Schultz Bip. ssp. *sivasicum* Hub.-Mor. & Grierson, *T. densum* (Lab.) Schultz Bip. ssp. *eginense* Heywood and *T. densum* (Lab.) Schultz Bip. ssp. *amani* Heywood which all are endemic to Turkish flora were investigated by DPPH free radical scavenging and β-carotene/linoleic acid assays. According to the results, the most active plant was found *T. densum* ssp. *amani* with an IC_{50} value of 69.30 μg/mL in DPPH system and *T. densum* ssp. *sivasicum* with an 79.10% inhibition ratio in β-carotene/linoleic acid system. The amount of total phenolics was highest in ssp. *sivasicum* (162.33 μg gallic acid equivalents/mg extract), which was followed by ssp. *amani* (158.44 μg gallic acid equivalents/mg extract). The results of this study revealed a positive correlation between total phenolic content and antioxidant activity of the extracts (Tepe and Sokmen, 2007). Similar correlation was also found by our study but in lower levels with respect to the results of Tepe and Sokmen. The antioxidant activity of ethanolic extracts of six *Tanacetum* species (*T. budjnurdense* (Rech.f.) Tzvelev, *T. hololeucum* (Bornm.) Podlech, *T. chiliophyllum* (Fisch.&Mey.) Sch.Bip., *T. sonbolii* Mozaff., *T. tabrisianum* (Boiss.) Sosn.&Takht., *T. kotschyi* (Boiss.) Grierson) from Iran was also examined by various test systems including DPPH (Among these taxa; *T. chiliophyllum, T. tabrisianum* and *T. kotschyi* grows naturally in Turkey). According to the results, all *Tanacetum* extracts showed antioxidant activity with IC_{50} values ranging from 59.55 to 157.24 μg/mL. Total phenolic contents of extracts range between 28.90 and 47.11 mg gallic acid equivalents/g extract and the total flavonoid contents were between 14.32 and 40.32 mg catechin equivalents/g extract. The extract of *T. hololeucum* which had the highest total phenolic content, was more active than other *Tanacetum* extracts (Esmaeili et al., 2010). In our study, *Tanacetum* extracts (*T. armenum, T. cadmeum* ssp. *cadmeum, T. cilicicum, T. praeteritum* ssp. *massicyticum, T. praeteritum* ssp. *praeteritum*) showed DPPH scavenging activity at higher IC_{50} values ranging from 197.82 to 247.57 μg/mL. On the other hand, total phenolic contents of the taxa we investigated were generally higher (in range between 33.14 and 149.93 mg gallic acid equivalents/g extract); total flavonoid contents were nearly at equal levels (in range between 7.69 and 33.42 mg quercetin equivalents/g extract) in comparison to the results of Esmaeili and his friends.

CONCLUSION

In this study, antioxidant activity of methanol extracts of the aerial parts of five *Tanacetum* taxa growing in Antalya was evaluated for the first time by using four methods which differ from each other by reaction, probes, quantitation manner, mechanisms or conditions. The results demonstrate that all plant extracts have antioxidant activity. Among these taxa *T. praeteritum* ssp. *massicyticum* which has the highest total phenolic and flavonoid contents comes to the forefront also by exhibiting the highest antioxidant activity. It is an endemic species and studying with the endemic species may be of great importance, because their bioactive features could be lost forever without being tapped (Esmaeili et al., 2010).

For the methanol extracts of *Tanacetum* taxa, the DPPH, ABTS, CUPRAC and FRAP assays revealed comparable results in this study. All of these techniques generally showed high reproducibility, were simple, easily performed and usually showed the predictable correlation with both total phenolic and flavonoid contents. Therefore, they would be the appropriate techniques for determining antioxidant potencies of plant extracts. Also all in all, antioxidant activity measured in methanol extracts may also be estimated indirectly and approximately by using total phenolic/flavonoid contents since they showed acceptable correlation with all assays. On the other hand, the chemical profile and structures of components directly contribute to the potential of natural antioxidants. Describing the antioxidant activity of plant extracts couldn't be done merely on the basis of their total phenolic content, which also needs their characterization. Different activity manners of the phenolics present in the extracts cannot be ignored. In conclusion, it is clear that more scientific work needs to be done for the isolation and identification of substantial phenolic compounds and for better understanding of their mechanism of action as antioxidant by *in vivo* experiments.

ACKNOWLEDGMENT

We would like to thank to Scientific Research Projects Foundation of Ankara University for the financial support provided through the Project 13L3336007.

REFERENCES

Abad, M. J., Bermejo, P., & Villar, A. (1995). An approach to the genus *Tanacetum* L. (Compositae): Phytochemical and pharmacological review. *Phytotherapy Research, 9*(2), 79-92.

Alam, M. N., Bristi, N. J., & Rafiquazzaman, M. (2013). Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal*, *21*, 143-152.

Antolovich, M., Prenzler, P. D., Patsalides, E., McDonald, S., & Robards, K. (2002). Methods for testing antioxidant activity. *The Royal Society of Chemistry, 127*, 183-198.

Apak, R., Güçlü, K., Özyürek, M., & Karademir, S. E. (2004). A novel total antioxidant capacity index for dietary polyphenols, vitamin C and E, using their cupric ion reducing capability in the presence of neocuproine: The CUPRAC method. *Journal of Agricultural and Food Chemistry, 52*(26), 7970-7981.

Badarinath, A. V., Rao, K. M., Chetty, C. M. S., Ramkanth, S., Rajan, T. V. S., & Gnanaprakash, K. (2010). A review on *in-vitro* antioxidant methods: Comparisions, correlations and considerations. *International Journal of PharmTech Research, 2*(2), 1276-1285.

Baranauskienė, R., Kazernavičiūtė, R., Pukalskienė, M., Maždžierienė, R., & Venskutonis P. R. (2014). Agrorefinery of *Tanacetum vulgare* L. into valuable products and evaluation of their antioxidant properties and phytochemical composition. *Industrial Crops and Products, 60*, 113-122.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology, 28*(1), 25-30.

Chang, C., Yang, M., Wen, H., & Chern, J. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis, 10*(3), 178-182.

Esmaeili, M. A., Sonboli, A., & Noushabadi, M. A. (2010). Antioxidant and protective properties of six *Tanacetum* species against hydrogen peroxide-induced oxidative stress in K562 cell line: A comparative study. *Food Chemistry, 121*, 148-155.

Esmaeili, A., & Amiri, H. (2011). The *in vitro* antioxidant and antibacterial activities of *Tanacetum pinnatum* boiss. grown in Iran. *Bulgarian Chemical Commuications, 43*(4), 532-537.

Gecibesler, I. H., Kocak, A., & Demirtas, I. (2016). Biological activities, phenolic profiles and essential oil components of *Tanacetum cilicicum* (Boiss.) Grierson. *Natural Product Research, 30*(24), 2850-2855.

Gören, N., Arda, N., & Çalışkan, Z. (2002). Chemical characterization and biological activities of the genus *Tanacetum* (Compositae). In R. Atta ur (Eds.), *Studies in Natural Products Chemistry* (pp. 547-658). Elsevier.

Grierson, A. (1975). *Tanacetum* L. In P. H. Davis (Eds.), *Flora of Turkey and the East Aegean Islands* (Vol. 5, pp. 256-292). Edinburgh: Edinburgh University Press.

Güner, A., Aslan, S., Ekim, T., Vural, M., & Babaç, M. (2012). *Türkiye Bitkileri Listesi (Damarlı Bitkiler)*. İstanbul: ANG Vakfi, Nezahat Gökyiğit Botanik Bahçesi Yayınları.

Ivanova, D., Gerova, D., Chervenkov, T., & Yankova, T. (2005). Polyphenols and antioxidant capacity of Bulgarian medicinal plants. *Journal of Ethnopharmacology, 96*, 145-150.

Kähkönen, M. P., Hopia, A. I., Vuorela, H. J, Rauha, J-P., Pihlaja, K., Kujala, T. S., & Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry, 47*, 3954-3962.

Krishnaiah, D., Sarbatly, R., & Nithyanandam, R. (2011). A review of the antioxidant potential of medicinal plants species. *Food and Bioproducts Processing, 89*(3), 217-233.

Minkwitz, E. (1999). *Over-the-Counter Nutritional Supplements: U.S. Market Trends and Key Players*. Decision Resources.

Miser-Salihoğlu, E., Akaydın, G., Çalışkan-Can, E., & Yardım-Akaydın, S. (2010). Evaluation of antioxidant activity of various herbal folk evaluation medicine. *FABAD Journal of Pharmaceutical Sciences, 35*, 59-67.

Oyaizu, M. (1986). Studies on product of browning reaction prepared from glucose amine. *Japanese Journal of Nutrition, 44*, 307-315.

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

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*(9), 1231-1237.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Sciences, 2*(4), 152-159.

Sacchetti, G., Maietti, S., Muzzoli, M., Scaglianti, M., Manfredini, S., Radice, M., & Bruni, R. (2005). Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. *Food Chemistry, 91*, 621-632.

Şenol, F. Z., Orhan, I., Celep, F., Kahraman, A., Doğan, M., Yilmaz, G., & Şener, B. (2010). Survey of 55 Turkish *Salvia* taxa for their acetylcholinesterase inhibitory and antioxidant activities. *Food Chemistry, 120*, 34-43.

Shahidi, F., & Zhong, Y. (2015). Measurement of antioxidant activity. *Journal of Functional Foods, 18*, 757-781.

Siti, H. N., Kamisah, Y., & Kamsiah, J. (2015). The role of oxidative stress, antioxidants and vascular inflammation in cardiovascular disease (a review). *Vascular Pharmacology*, *71*, 40-56.

Slinkard, K., & Singleton, V. L. (1977). Total phenol analysis: Automation and comparison with manual methods. *American Journal of Enology and Viticulture, 28*(1), 49-55.

Sonboli, A., Stroka, K., Kazempour Osaloo, S., & Oberprieler, C. (2012). Molecular phylogeny and taxonomy of *Tanacetum* L. (Compositae, Anthemideae) inferred from nrDNA ITS and cpDNA trnH–psbA sequence variation. *Plant Systematics and Evolution, 298*(2), 431-444.

Tawaha, K., Alali, F. Q., Gharaibeh, M., Mohammad, M., & El-Elimat, T. (2007). Antioxidant activity and total phenolic content of selected Jordanian plant species, *Food Chemistry, 104*, 1372-1378.

Tepe, B., & Sokmen, A. (2007). Screening of the antioxidative properties and total phenolic contents of three endemic *Tanacetum* subspecies from Turkish flora. *Bioresource Technology, 98*(16), 3076-3079.

Wojdyło, A., Oszmiański, J., & Czemerys, R. (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chemistry, 105*, 940-949.

Wojtunik-Kulesza, K. A., Oniszczuk, A., Oniszczuk., T., & Waksmundzka-Hajnos, M. (2016). The influence of common free radicals and antioxidants on development of Alzheimer's Disease. *Biomedicine & Pharmacotherapy, 78*, 39-49.

Wu, C., Chen, F., Wang, X., Kim, H-J., He, G-Q., Haley-Zitlin, V., & Huang, G. (2006). Antioxidant constituents in feverfew (*Tanacetum parthenium*) extract and their chromatographic quantification. *Food Chemistry, 96*(2), 220-227.