Antibacterial, anticandidal and antioxidant properties of *Tanacetum argenteum* (Lam.) Willd. subsp. *flabellifolium* (Boiss. & Heldr.) grierson

Yavuz Bulent Kose[1]*, Gokalp Iscan[2, 3], Fatih Goger[2], Betul Demirci[2] and Ceren Elmacı[4]

[1]Faculty of Pharmacy, Department of Pharmaceutical Botany, Anadolu University, Eskisehir, Turkey
[2]Faculty of Pharmacy, Department of Pharmacognosy, Anadolu University, Eskisehir, Turkey
[3]Yunus Emre Vocational School, Anadolu University, Eskisehir, Turkey
[4]Institute of Science, Department of Advanced Technologies, Anadolu University, Eskisehir, Turkey

Abstract: In the present study hydrodistilled essential oil and total methanol extracts of *Tanacetum argenteum* subsp. *flabellifolium* have been evaluated for their antimicrobial and antioxidant effects. The chemical composition of the oil and the crude extract were determined by GC/FID, GC/MS and LC/DAD/ESI-MS systems respectively. β-thujone (47.1%), α-pinene (19.1%) and α-thujone (10.5%) were the main compounds of the essential oil while the 5-*O*-caffeoylquinic acid, 1,5-*O*-dicaffeoylquinic acid, 4,5-*O*-dicaffeoylquinic acid were identified as flavonoid content of the crude extract. The oil and the methanol extract were demonstrated moderate antimicrobial effects (MIC range; 0,062-2,0 mg/mL) against 21 different pathogenic micro organism. Total phenolic content was determined as 63 mg GAE in g extract and the DPPH radical scavenging effect was determined as 0.16 mg/mL (IC_{50}) and TEAC was determined as 0.21mMol.

Keywords: *Tanacetum argenteum* subsp. *flabellifolium,* essential oil, methanol extract, antimicrobial, antioxidant, GC-FID, GC/MS, LC-MS/MS.

INTRODUCTION

Tanacetum L., the third biggest genus of Asteraceae (Compositae)-Anthemideae, is represented with about 160 species all over the world 46 of them are found in Turkey. (Sonboli *et al*., 2012, Güner, 2012). There are three subspecies of *T. argenteum* (Lam.) Willd. in Turkey: *argenteum, flabellifolium* (Boiss. & Heldr.) Grierson and *canum* (C. Koch) Grierson (Gören *et al*., 2001). *Tanacetum* species are rich in phenolics, pyrethrins, bitter substances, essential oils, and sesquiterpene lactones and they are widely used in folk medicine for their antihistaminic, anti-inflammatory and insecticidal effects (Baser *et al*., 2001, Marongiu *et al*., 2009, Baranauskienė *et al*., 2014,). Well-known Feverfew (*T. parthenium*) is a species that is widely used against migraine due to the presence of the sesquiterpene lactones and flavonoids (Chaves *et al*., 2009). Antimicrobial drug searching derived from plant origin has undergone great development in recent years. Generally, plants produce secondary metabolites for defense against microorganisms, insects, etc. Apart from flavor and fragrance properties, essential oils can be used in the agriculture, food, cosmetic and pharmaceutical industries for their antibacterial, antifungal and antioxidant properties (Cowan, 1999). The herbal infusion of *Tanacetum argenteum* is used for pain reliever in Anatolian folk medicine for its analgesic properties (Akan *et al*., 2013). In the current study the chemical composition of *T. argenteum* subsp. *flabellifolium* have been determined by chromato-spectrometric methods. Furthermore, hydro distilled essential oil and methanol extract of the flowering aerial parts were evaluated for their biological activity.

**Corresponding author:* e-mail: ybkose@anadolu.edu.tr

MATERIALS AND METHODS

Plant material

Plant samples collected from Turkey, İçel, Anamur-Ermenek road, Suolmaz pass, 36°16'32"N 32°54'54"E, 1650 m, 19 VI 2014. ESSE No: 14798.

Isolation of essential oil

The essential oil was isolated by hydro distillation for 3 h from air-dried aereal parts of *T. argenteum* subsp. *flabellifolium*. Hydro distillation was performed by using a Clevenger-type apparatus according to the method recommended in the European Pharmacopoeia (2010). A small amount of essential oil trapped in *n*-hexane was collected for oil analysis.

MeOH extraction

Powdered dried herbal parts of *T. argenteum* subsp. *flabellifolium* were macerated with methanol 70% at 25 °C for 24h. After evaporation of the methanol part, the aqueous part freeze-dried and the dry extract was used for in all experiments.

GC-MS and GC analysis

GC and GC-MS analysis were performed according to our previously published work (Ali *et al*., 2015). The analysis results are given in table 1.

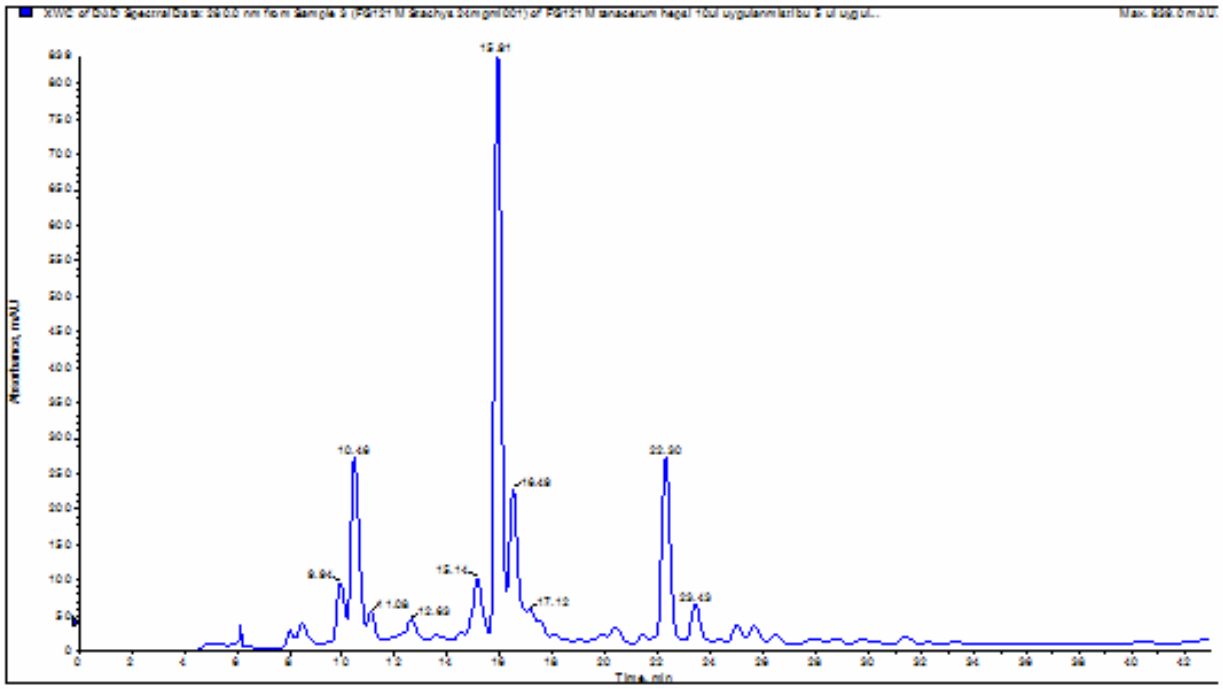

Fig. 1: *T. argenteum* LC chromatogram at 280 nm.

Determination of the essential oil constituents were accomplished by comparison of relative retention times with authentic samples or relative retention index (RRI) to series of *n*-alkanes. Additionallycomputer matching was used against commercial libraries (Wiley GC/MS Library, Mass Finder 3 Library) (Mc Lafferty and Stauffer, 1989; Koenig, 2004) and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils and several literature MS data (Joulain and Koenig, 1998; ESO 2000, 1999).

Antimicrobial susceptibility tests

Antibacterial and anticandidal effects of the essential oil and crude methanolic extract of *T. argenteum* subsp. *flabellifolium* were screened by using partly modified CLSI (formerly NCCLS) microdilution broth methods M7-A7 (Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically) and M27-A2 (Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts) respectively (CLSI (NCCLS) M7-A7, 2006; CLSI (NCCLS) M27-A2, 2002).

For checking purity and viability *Candida* and bacterial strains were inoculated from the -85°C onto potato dextrose agar (Fluka) and Mueller Hinton agar (Fluka) respectively. All microdilution broth tests were performed by using sterile 96 U-shaped multiwell microdilution plates (L.P. Italiana) in laminar flow cabinet. The MIC data from anticandidal tests was obtained after 24h. Furthermore in M27-A2 method, *C. parapsilosis* (ATCC® 22019) and *C. krusei* (ATCC® 6258) were used as control microorganisms. To precision and accuracy of the susceptibility tests procedure, the MIC results of the standard antimicrobial agents against QC strains were checked from the CLSI-MIC limits tables. Ampicillin and chloramphenicol were used as antibacterial agents where the Amphotericin-B and Ketoconazole were used as antifungal.

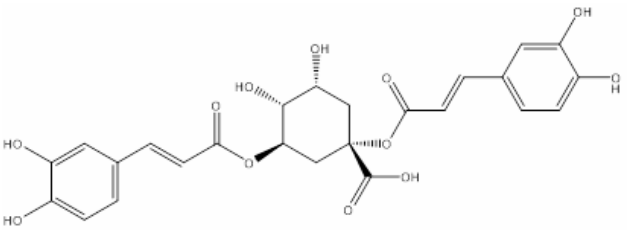

Fig. 2: 1,5-Dicafeoylquinic acid (cynarine), the main compound of the MeOH extract.

Antioxidant activity (total phenolics)

Total phenols were estimated as Gallic acid equivalents (GAE), expressed as mg Gallic acid/g extract. (Singleton, 1999)

TEAC assay (trolox equivalent antioxidant capacity)

This assay was performed according to previously described literature (Papandreou *et al*., 2006).

Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

A microplate microdilution method, previously described Kumarasamy and colleagues, was used for the determination of radical scavenging activity (Kumarasamy *et al*., 2007).

Table 1: The composition of the essential oil of *T. argenteum* subp. *Flabellifolium*

RRI	Compound	%	Identification method
1032	α-Pinene	19.1	t_R, MS
1035	α-Thujene	1.0	MS
1118	β-Pinene	0.5	t_R, MS
1132	Sabinene	2.0	t_R, MS
1213	1,8-Cineole	0.3	t_R, MS
1255	γ-Terpinene	0.3	t_R, MS
1280	*p*-Cymene	0.2	t_R, MS
1285	Isoamyl isovalerate	0.2	MS
1437	α-Thujone	10.5	MS
1451	β-Thujone	47.1	MS
1474	*trans*-Sabinene hydrate	0.3	MS
1586	Pinocarvone	2.2	t_R, MS
1611	Terpinen-4-ol	0.7	t_R, MS
1612	β-Caryophyllene	0.9	t_R, MS
1658	Sabinyl acetate	2.1	MS
1670	*trans*-Pinocarveol	2.1	t_R, MS
1683	*trans*-Verbenol	0.9	t_R, MS
1687	α-Humulene	0.2	t_R, MS
1704	Myrtenyl acetate	0.2	MS
1706	α-Terpineol	0.2	t_R, MS
1720	*trans*-Sabinol	1.5	MS
1804	Myrtenol	0.2	MS
1845	*trans*-Carveol	0.1	t_R, MS
1866	Methyl hydrocinnamate	0.2	MS
1969	*cis*-Jasmone	0.3	MS
2008	Caryophyllene oxide	1.4	t_R, MS
2071	Humulene epoxide-II	0.4	MS
2324	Caryophylla-2(12),6(13)-dien-5 α-ol (=*Caryophylladienol II*)	0.3	MS
	Total	**95.4**	

RRI Relative retention indices calculated against *n*-alkanes % calculated from FID data, Identification method: t_R, identification based on the retention times (t_R) of genuine compounds on the HP Innowax column; MS, identified on the basis of computer matching of the mass spectra with those of the Wiley and MassFinder libraries and comparison with literature data.

Table 2: Compounds determined with LC-MS/MS

No	Compound	Rt	(M-H)	Fragments	Refence
1	Quinic acid	5.8	191	85(100)	(Clifford *et al*., 2005)
2	5-Caffeoylquinic acid	10.5	353	353(16), 191(100)	(Clifford *et al*., 2003)
3	1,5-*O*-Dicafeoylquinic acid	15.9	515	515(23), 191(100), 335(5)	(Clifford *et al*., 2005)
4	4,5-*O*-Dicafeoylquinic acid	16.5	515	161(9), 179(12), 353(35), 135 (18)	(Clifford *et al*., 2005)
5	Nepetin	22.3	315	515(17), 191(39), 173(100), 353(17)	(Baranauskienė *et al*., 2014)
6	Axillarin	23.5	345	300(100), MS^2 201(80), 133 (100)	(Baranauskienė *et al*., 2014)
7	Apigenin	24.8	269	330(100), 315(39)	(Baranauskienė *et al*., 2014)

Phenolic compound determination

Experiments were performed with a Shimadzu 20A HPLC system coupled to an Applied Biosystems 3200 Q-Trap LC- MS/MS instrument equipped with an ESI ion source was used in the negative ionization mode. Separations were performed on an ODS 150 x 4,6 mm, i.d., 3µm particle sizes, octadecyl silica gel analytical column operating at 40ºC at a flow rate of 0.3 ml/min. The mobile phase was used Methanol: Water: Formic acid (10:89:1) (solvent A) and Methanol: Water: Formic acid (89:10:1) (solvent B). The composition of B was increased from 15% to 40% in 15min, increased to 45% in 3 min and held for 12 min, and increased to 75% in 5 min; then the composition of B was increased to 100% in 10 min. (fig. 1).

RESULTS

The essential oil components of *T. argenteum* subsp. *flabellifolium* were given in table 1 with their relative

Table 3: Antibacterial results of *T. argenteum* subsp. *flabellifolium* Essential oil and Methanolic extract (µg/mL, MIC)

Strains		EO	ME	St1	St2
Gram (-)					
Pseudomonas aeruginosa	ATCC 27853	1000	500	64	8
Enterobacter aerogenes	NRRL 3567	1000	500	32	2
Proteus vulgaris	NRRL B-123	125	250	1	4
Salmonella typhimurium	ATCC 14028	500	2000	1	1
Escherichia coli	ATCC 8739	2000	500	2	1
E. coli O157:H7	RSSK 234	500	1000	2	1
Serratia marcescens	NRRL B-2544	500	500	32	4
Gram (+)					
Bacillus cereus	NRRL B-3711	125	125	2	1
Bacillus subtilis	NRRL B-4378	250	250	1	1
Staphylococcus aureus	ATCC 43300	125	250	1	8
Listeria monocytogenes	ATCC 19111	500	500	1	4
Staphylococcus epidermidis	ATCC 14990	1000	500	1	2

EO: Essential oil, ME: Methanolic extract, St1: Ampicilline, St2: Chloramphenicol, RSSK: Refik Saydam National Type Culture Collection

Table 4: Anticandidal results of *T. argenteum* subsp. *flabellifolium* Essential oil and Methanolic extract (µg/mL, MIC)

Strains		EO	ME	St1	St2
Candida albicans[1]	ATCC 10231	500	250	0.5	0.5
Candida albicans[2]	ATCC® 24433	1000	500	0.5	1
Candida parapsilosis[1]	NRRL Y- 12696	250	62	32	16
Candida parapsilosis[2]	ATCC 22019	2000	250	0.5	0.5
Candida utilis	NRRL Y-900	1000	62	0.5	0.5
Candida zeylanoides	NRRLY-1774	250	62	8	4
Candida krusei[1]	NRRL Y-7179	1000	31	0.5	0.5
Candida krusei[2]	ATCC 6258	1000	62	1	0.5
Candida glabrata[1]	ATCC 2001	2000	125	0.5	0.5
Candida glabrata[2]	ATCC 66032	2000	1000	2	0.5
Candida tropicalis[1]	ATCC 1369	2000	500	0.5	0.5
Candida tropicalis[2]	ATCC 750	500	250	1	0.5

EO: Essential oil, ME: Methanolic extract, St1: Amphotericin-B, St2: Ketoconazole

percentages. 28 compounds, representing 95.4% of the oil, were identified, with β-thujone (47.1%), α-pinene (19.1%) and α-thujone (10.5%) as the main constituents. Previous investigation reported that essential oil composition of *T. argenteum* subsp. *flabellifolium* with different main components. According to this report α-pinene (29.1%), (*E*)-sesquilavandulol (15.9%) and camphor (14.0%) were found as main constituents (Tabanca *et al*., 2007).

The essential oil and the crude methanolic extract of *T. argenteum* subsp. *flabellifolium* were screened for their antimicrobial activities by using micro dilution methods against 24 pathogenic bacteria and *Candida* species. The essential oil demonstrated weak to moderate effects against both *Candida* and Bacteria panel having MIC values between 125 to 2000mg/mL. Methanol extract remarkably showed strong inhibition effects against *Candida* species, especially *C. krusei* (NRRL Y-7179 and ATCC 6258), *C. parapsilosis* (NRRL Y- 12696), *C. utilis* and *C. zeylanoides* at the concentrations of 31 and 62 mg/mL (table 3).

According to LC-MS/MS analysis, (table 2) MeOH extract was found as rich for caffeoylquinic acids derivatives, which were determined using the fragmentation behavior, previously described Cliford and his colleagues (Clifford *et al*., 2003, Clifford *et al*., 2005). 1, 5-Dicafeoylquinic acid, known as cynarine, was determined as main compound (fig. 2). Other compounds were determined using the literature data previously reported about *Tanacetum vulgare* phenolics by Baranauskienė and colleagues (Baranauskienė *et al*., 2014).

Antioxidant activity of the extract showed low activity due to the low phenolic content value, which was found 63±2 mgGAE in g extract. Extract was found ten times

weaker than the positive control Gallic acid: DPPH IC_{50}=0.16±0.02mg/ml while it was determined 0.02 mg/ml for positive control gallic acid, TEAC =0.21 mMol TEAC while it is determined 2.47 for positive control Gallic acid. It is not possible to determine any antioxidant activity for essential oil.

DISCUSSION

The essential oils isolated from *Tanacetum* species have variable chemical constituents. Chemovariation is a well-known situation for *Tanacetum* species (Kumar & Tyagi, 2013). These results allow the assignments the present species is thujone chemotype. Essential oils including thujone are being used in traditional medicine for the abortifacient, female hormone activity, emmenagogue, and anthelmintic (Siveen & Kuttan, 2011).

Anticandidal activity is probably due to lipophilic flavonoids and terpenoids that may also disrupt microbial membranes. The bioactivity results showed a promising anticandidal activity of Methanolic extract of the *T. argenteum* subsp. *flabellifolium* (table 4).

Previously reported data about antimicrobial investigation *of T. argenteum* subsp. *Argenteum* essential oil (Polatoglu *et al*., 2010) confirmed some of our results that *Bacillus cereus* and *Bacillus subtilis* showed Inhibiton at concentration 125 µg/mL (table 4). Tabanca and colleagues showed minimum inhibitory concentration of the essential oil four times more effective againts *Candida albicans* than our results. It could be explained by essential oil composition. Tabanca and colleagues identified the main compound as α-Pinene (Tabanca *et al*., 2007) while it was found as β–Thujone in our presented study.

In consequence of this finding, as far as we know, the antimicrobial and antioxidant activity were determined the first time for *T. argenteum subsp. flabellifolium* extract, especially against *Candida* species. We determined that it has low antioxidant activity but strong antifungal activity. In further studies active fractions of the extracts can be determined and more active components can be obtain against to other *Candida, Cryptococcus and Aspergillus* species for using as an alternative treatment.

REFERENCES

Akan H, Aydogdu M, Korkut, MM and Balos MM (2013). An ethnobotanical research of the Kalecik mountain area (Şanlıurfa, South-East Anatolia). *Bio. Div. and Cons.*, **6**(2): 84-90.

Ali A, Tabanca N, Demirci B, Blythe EK, Ali Z, Başer KHC and Khan IA (2015). Chemical composition and biological activity of four Salvia essential oils and individual compounds against two species of mosquitoes. *J. Agric. Food Chem*., **63**: 447-456.

Baser KHC, Demirci B, Tabanca N, Ozek T and Goren N (2001). Composition of the essential oils of *Tanacetum armenum* (DC.) Schultz Bip., *T. balsamita* L., *T. chiliophyllum* (Fisch. &Mey.) Schultz Bip. var. *chiliophyllum* and *T. haradjani* (Rech. fil.) Grierson and the enantiomeric distribution of camphor and carvone. *Flavour. Fragr. J.,* **16**: 195-200.

Baranauskienė R, Kazernaviciute R, Pukalskienė M, Maždžierienė R and Venskutonis PR (2014). Agrorefinery of Tanacetum vulgare L. into valuable products and evaluation of their antioxidant properties and phytochemical composition. *Ind. Crop. Prod.,* **60**: 113-122.

Chaves JS, Da Costa FB and de Freitas LAP (2009). Development of enteric coated tablets from spray dried extract of feverfew (Tanacetumparthenium L.). *Brazilian J. of Pharm. Sci.*, **45**(3): 573-584.

Clifford MN, Johnston KL, Knight S and Kuhnert N (2003). Hierarchical scheme for LC-MS n identification of chlorogenic acids. *J. Agr. Food. Chem.,* **51**: 2900-2911.

Clifford, MN, Knight S and Kuhnert N(2005).Discriminating between the six isomers of dicaffeoylquinic acid by LC-MS n. *J. Agr.Food. Chem.,* **53**: 3821-3832.

CLSI (NCCLS) M7-A7 (2006).Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard, Seventh Edition.

CLSI (NCCLS) M27-A2(2002).Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard, Second Edition.

Cowan MM (1999). Plant products as antimicrobial agents. *Clin. Microbiol. Rev.,* **12**(4): 564-82.

ESO 2000 (1999).The Complete Database of Essential Oils, Boelens Aroma Chemical Information Service, The Netherlands.

European Pharmacopoeia (2010).Council of Europe 7th ed., European Directorate for the Quality of Medicines & HealthCare, Strasbourg.

Gören N, Demirci B and Başer KHC (2001). Composition of the essential oils of Tanacetum spp. from Turkey. *Flavour Fragr J.*, **16**(3): 191-194.

Güner A. (ed.) Turkiye Bitkileri Listesi (2012). Ali Nihat Gökyiğit Vakfı Yayınları, İstanbul, Turkey.

Joulain D and Koenig WA (1998). The Atlas of Spectra Data of Sesquiterpene Hydrocarbons, EB-Verlag, Hamburg.

Koenig WA, Joulain D and Hochmuth DH (2004). Terpenoids and Related Constituents of Essential Oils. Mass Finder 3, Hamburg, Germany.

Kumar V and Tyagi D (2013). Chemical Composition and Biological Activities of Essential Oils of Genus *Tanacetum* - a review. *J. of Pharmacognosy and Phytochem.*, **2**(3): 159-163.

Kumarasamy Y, Byres M, Cox PJ, Jaspars M, Nahar L and Sarker SD (2007). Screening seeds of some Scottish plants for free radical scavenging activity. *Phytother. Res.,* **217**: 615-621.

Marongiu B, Piras A, Porcedda S, Tuveri E, Laconi S, Deidda D and Maxia A (2009). Chemical and biological comparisons on supercritical extracts of *Tanacetum cinerariifolium* (Trevir) Sch. Bip. with three related species of chrysanthemums of Sardinia (Italy). *Nat. Prod. Res.*, **23**(2): 190-199.

McLafferty FW and Stauffer DB (1989). The Wiley/NBS Registry of Mass Spectral Data, J Wiley and Sons: New York.

Papandreou MA, Kanakis CD, Polissiou MG, Efthimiopoulos S, Cordopatis P, Margarity M and Lamari FN (2006). Inhibitory activity on amyloid-β aggregation and antioxidant properties of Crocus sativus stigmas extract and its crocin constituents. *J. Agr. Food. Chem.,* **5423**: 8762-8768.

Polatoglu K, Demirci F, Demirci B, Gören N and Baser KHC (2010). Essential oil composition and antibacterial activity of Tanacetum argenteum (Lam.) willd. ssp. argenteum and T. densum (Lab.) Schultz Bip. ssp. Amani Heywood from Turkey. *J. Oleo Sci.,* **59**: 361-367.

Tabanca N, Demirci F, Demirci B, Wedge DE and Baser KHC (2007). Composition, enantiomeric distribution, and antimicrobial activity of *Tanacetum argenteum* subsp. *flabellifolium* essential oil. *J. Pharmaceut. Biomed.*, **45**: 714-719.

Singleton Vernon L, Orthofer R and Lamuela-Raventos RM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol*., **299C**: 152-178.

Siveen KS and Kuttan G (2011). Augmentation of humoral and cell mediated immune responses by Thujone, *Int. Immunopharm*., **11**: 1967-1975.

Sonboli A, Stroka K, Osaloo SK and Oberprieler C (2012). Molecular phylogeny and taxonomy of *Tanacetum* L. (Compositae, Anthemideae) inferred from nrDNA ITS and cpDNA trnH-psbA sequence variation. *Plant. Syst. Evol*., **298**: 431-444.