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Phytochemical Contents of Five *Artemisia* Species

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Abstract

In the present study, the fatty acid compositions, vitamin, sterol contents and flavonoid constituents of five Turkish *Artemisia* species (*A. armeniaca*, *A. incana* , *A. tournefortiana, A. haussknechtii* and *A. scoparia*) were determined by GC and HPLC techniques. The results of the fatty acid analysis showed that *Artemisia* species possess high saturated fatty acid compositions. On the other hand, the studied *Artemisia* species were found to have low vitamin and sterol contents. Eight flavononids (catechin, naringin, rutin, myricetin, morin, naringenin, quercetin, kaempferol) were determined in the present study. It was found that *Artemisia* species contained high levels of flavonoids. Morin (45.35 ± 0.65 – 1406.79 ± 4.12 µg/g) and naringenin (15.32 ± 0.46 – 191.18 ± 1.22 µg/g) were identified in all five species. Naringin (268.13 ± 1.52 – 226.43 ± 1.17 µg/g) and kaempferol (21.74 ± 0.65 – 262.19 ± 1.38 µg/g) contents were noted in the present study. Present research showed that the studied *Artemisia* taxa have high saturated fatty acids and also rich flavonoid content.

Keywords: *Artemisia*, fatty acid, flavonoid, sterol, vitamin

Introduction

Artemisia L. is the largest genus in the tribe *Anthemideae* of the *Asteraceae*, which comprises 400-500 species (Martin *et al.,* 2001; Kreitschiz and Valles, 2007). Most of these species grow wild in dry or semi-dry habitats throughout the Northern regions of the world (Torrell and Valles, 2001; Valles *et al.,* 2003; Singh *et al.,* 2009). The genus is composed of perennial, biennial and annual herbs or small shrubs, which are frequently aromatic (Kordali *et al.,* 2005; Kursat, 2010). There are 23 A*rtemisia* species in the flora of Turkey, which are distributed throughout the country (Davis, 1975).

In the traditional herbal medicine, aerial parts of *Artemisia* are being used for its antimalarial, antimicrobial, antioxidant, antifungal, anticholesterolemic, antidiabetic, antihelminth, antiseptic, antitumor, antipyretic and antispasmodic effects (Chopra *et al*., 1992; Koul, 1997; Cimbiz et al., 2005; Han *et al.,* 2007; Temraz and El-Tantawy, 2008; Sengul *et al.,* 2011; Afshar *et al.,* 2013). Several studies showed that polyphenols, flavonoids, cinnamic acid derivatives and coumarines were found in *Artemisia* species (Esteban *et al.,* 1986; Rauter *et al.,* 1989; Mojarrab *et al.,* 2011; Afsar *et al.,* 2012; Suresh *et al.,* 2012). Plant phenolics are widespread natural compounds and are probably responsible for the antioxidant activity by thier hydroxyl groups, and thus have the ability to neutralize free radicals (Fukumato and Mazza, 2000; Ruikar *et al.,* 2011; Bandli and Heidari, 2014).

Natural and cultivated Turkish herbs are being more widely used on a commercial scale in the food industry and in traditional medicine (Baytop, 1999; Erdemoglu *et al.,* 2007). To support the use of herbal medicine and to detect their potential as possible drugs it is necessary to work medicinal plants (Sengul *et al.,* 2011). The aim of the present study was to establish possible medicinal properties of some *Artemisia* species by determining the fatty acid compositions, vitamin and sterol contents, flavonoid constituents of aerial parts, of five *Artemisia* species growing in Turkey.

Materials and Methods

Chemicals

All chemicals, solvents and standards were purchased from Sigma-Aldrich-Fluka (Taufkirchen, Germany) or Merck (Darmstadt, Germany).

Plant materials

The present study examined plant extracts of five *Artemisia* species: *A. armeniaca* Lam., *A. incana* (L.) Druce, *A. tournefortiana* Reichb., *A. haussknechtii* Boiss. and *A. scoparia* Waldst. & Kit. Sample plants were collected from their natural habitats. Details about the plant materials are shown in Table 1. Voucher specimens of studied plants were deposited at the Herbarium of Firat University (FUH). All experiments were performed in triplicate.

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Extraction of plant oils

Two g aerial parts of plant material were finely grounded in a mill and were then extracted with n-hexane/isopropanol (3:2 v/v) (Hara and Radin, 1978). The lipid extracts were centrifuged at 10,000 rpm for 5 min and filtered; the solvent was then removed on a rotary evaporator at 40 °C. The extracted lipids were stored under -25 °C until further analysis.

Fatty acid analyses

Fatty acid in the lipid extracts were converted into methyl esters by means of 2% sulphuric acid (v/v) in methanol (Christie, 1990). The fatty acid methyl esters were extracted with n-hexane. The methyl esters were then separated and quantified by gas chromatography and flame-ionization detection (Shimadzu GC 17 Ver.3) coupled to a CLASS GC 10 software computer software. Chromatography was performed with a capillary column (0.25 mm in diameter, Permabound 25, Macherey-Nagel, Germany) using nitrogen as a carrier gas (flow rate 0.8 ml/min.). The temperatures of the column, detector and injection valve were 130-220, 240 and 280 °C, respectively. Identification of the individual methyl esters was performed by frequent comparison with authentic standard mixtures that were analysed under the same conditions. Retention time for studied *Artemisia* species was determined as 3.34 min - *A. armeniaca*, 3.81 min - *A. incana*, 9.47 min - *A. tournefortiana*, 17.40 min - *A. haussknechtii*, 1.41 min - *A. scoparia*.

Chromatographic analysis and quantification of lipid soluble vitamins and sterols

Lipide-soluble vitamins and phytosterols were extracted from the lipid fraction by the method of Sánchez-Machado (Sánchez-Machado *et al.,* 2002) with minor modifications. The extracted lipids of plant material were dissolved in acetonitrile/methanol (75/25 v/v) and were injected 50 μL to HPLC UV detector (SPD-10AVP) instrument (Shimadzu, Kyota Japan). A Supelcosil LC18 (250 x 4.6 mm, 5 μm, Sigma, USA) column was used. The mobile phase was acetonitrile/methanol (75/25, v/v) and the elution was performed at a flow-rate of 1 ml/min The temperature of analytical column was kept at 40 °C. Detection was performed at 320 nm for retinol (vitamin A) and retinol acetate, and 215 nm for δ-tocopherol, vitamin D, α-tocopherol, α-tocopherol acetate, 202 nm for phytosterols, 265 nm for vitamin K1. Identification of the individual vitamins and phytosterols was performed by frequent comparison with authentic external standard mixtures (K2: 1.8 μg/5 ml; K1: 1.89 μg/5 ml; R-tocopherol: 1.84 μg/5 ml; D2: 2.14 μg/5 ml; D3: 2.25 μg/5 ml; α-tocopherol: 3.61 μg/5 ml; retinol: 1.4 μg/5 ml; retinol acetate: 3.14 μg/5 ml; ergosterol: 9.78 μg/5 ml; stigmasterol: 5.73 μg/5 ml; β-sitosterol: 3.1 μg/5 ml) analysed under the same conditions (López-Cervantes *et al.,* 2006). Retention time for studied *Artemisia* species was determined as 6.79 min (K2), 14.65 min (K1), 7.94 min (R-tocopherol), 8.82 min (D2), 9.46 min (D3), 10.23 min (α-tocopherol), 3.65 min (retinol), 3.95 min (retinol acetate), 11.57 min (ergosterol), 18.35 min (stigmasterol), 20.67 min (β-sitosterol).

Statistical analysis

Class Vp 6.1 software assisted at workup of the data. The results of analysis were expressed as μg/g for samples. Vitamine, sterol and flavonoid contents are given mean ± standard deviation as μg/g. Fatty acid compositions are given mean ± standart deviation (%).

Flavonoid analysis

Preparation of the extracts

Two g aerial parts of *Artemisia* were homogenized in 5 ml 80% methanol. Homogenates were centrifuged at 5,000 rpm at +4 °C. After centrifugation, the supernatant was concentrated by reduced-pressure rotary evaporation. Extract was re-suspended in 1 ml dimethyl sulphoxide (DMSO) to produce a stock solution.

Chromatographic conditions for flavonoids

Chromatographic analysis was carried out using a PREVAIL C18 reversed-phase column (15 x 4.6 mm, 5 μm, USA); the mobile phase was methanol/water/acetonitrile (46/46/8, v/v/v) containing 1.0% acetic acid (Zu *et al.*, 2006). The mobile phase was filtered through a 0.45 μm membrane filter (Millipore). Catechin (CA), naringin (NA), rutin (RU), myricetin (MYR), morin (MOR), naringenin (NAR), quercetin (QU) and kaempferol (KA) were quantified by DAD separation at 280 nm for CA and NA, 254 nm for RU, MYR, MOR and QU, and 265 nm for KA. Flow rate and injection volume were 1.0 ml/min and l0 μL, respectively. The chromatographic peaks of the extracts were confirmed by comparing their retention time with that of the reference standards. Quantification was carried out by the integration of the peak using the external standard method. All chromatographic operations were carried out at a temperature of 25 °C.

Results and Discussion

Fatty acid compositions, vitamin and sterol contents of Artemisia species

The fatty acid compositions of the studied *Artemisia* species were summarized in Table 2. The dominant fatty acids in the *Artemisia* species were found to be palmitic acid, stearic acid, palmitoleic acid, oleic acid, linoleic acid, eicosadienoic acid and docosadienoic acid. *A. armeniaca, A. haussknechtii* and *A. scoparia* contained the highest saturated fatty acid contents, at 65.21 ± 0.51%, 39.84 ± 0.49% and 58.01 ± 0.36%, respectively. Analysis by Orhan *et al.* (2009) of the fatty acid contents of different genus of *Asteraceae* suggested that most of the extracts seemed to be rich in saturated fatty acids.

Palmitoleic acid and oleic acid were found as monounsaturated fatty acids in all *Artemisia* species studied. *A. tournefortiana* and *A. haussknechtii* contained high levels of monounsaturated fatty acid (15.56 ± 0.28% and 20.58 ± 0.36%, respectively). Linoleic acid, eicosadienoic acid and docosadienoic acid were dominant polyunsaturated fatty acids in the five *Artemisia* species studied. Linolenic acid was absent or present at low levels in the studied species, except for *A. incana,* in which the linolenic acid content was found to be 22.21 ± 0.43%. Similarly, Orhan *et al.* (2009) found that the linoleic acid constituent of different species of *Asteraceae* was low or absent. Carvalho *et al.* (2011a) determined palmitic acid is major saturated fatty acid and linoleic acid and linolenic acids are major unsaturated fatty acids in the studied thirteen *Artemisia* species

The sterol and vitamin contents of the five *Artemisia* species studied are shown in Table 3. *A. armeniaca* had high ergosterol (164.75 ± 2.34 μg/g) and β-sitosterol (26.5 ± 1.23 μg/g) contents, but low stigmasterol content. The presence of sitosterol and ergosterol in *A. annua* was reported by Abid Ali Khan *et al.* (1991). Several previous studies indicated that phytosterols, such

as β-sitosterol, were most effective against reactive oxygen species (Vivacons and Moreno, 2005; Conforti *et al.,* 2009). Furthermore, the present study showed that the *Artemisia* species studied had low vitamin contents. Brisibe *et al.* (2009) reported that the vitamin A content of *A. annua* was below 0.3 g/100 g, while vitamin E was determined to be at high levels in *A. annua* leaves (22.63 mg/kg).

Flavonoid contents of Artemisia species

It was reported that phenolic compounds possess potent antioxidant activity and have anticancer or anti-carcinogenic/anti-mutagenic, antibacterial, antiviral or anti-inflammatory properties (Bo *et al*., 2002; Cai *et al*., 2006). The flavonoid content of *Artemisia* species are shown in Table 4. *Artemisia* species are generally known as rich sources of antioxidant such as flavonoids and coumarins (Toda, 2005; Brisibe *et al.,* 2009). The present study found that *A. armeniaca* had the highest flavonoid composition, while *A. haussknechtii* had less variety of flavonoid than other species studied. Mino *et al.* (2004) reported that *Artemisia* species included luteolin and kaempferol constituents. Also, Djeridane *et al.* (2006) concluded that all the plant species they studied including *Artemisia* species such as *Artemisia campestris, Artemisia herba-alba* and *Artemisia arborescens*- were rich in flavonoids. Furthermore, previous studies showed that different *Artemisia* species contain apigenin, luteolin, rutin, kaempferol, quercetin and naringenin constituents (Valant-Vetschera *et al.,* 2003; Cai *et al.,* 2004; Carvalho *et al.,* 2011b; Suresh *et al.,* 2012). Suresh *et al.* (2012) suggested that *Artemisia* species have significant amount of polyphenols and flavonoids content. Therefore these species could be considered in the category of antioxidant, anticancer, antimicrobial and immunomodulatory. Also, Carvalho *et al.* (2011b) found total phenolic content between 0.22-0.39 mg/gGAE in the leaves of *Artemisia* species. Also they determined the kaempherol the major flavonoid in the six *Artemisia* leaves, which measured in higher amounts 47.56 µg/g. However, quercetin and myricetin were determined in much lower quantities in the six *Artemisia* species (Carvalho *et al.*, 2011b). Sengul *et al.* (2011) found that total phenolic content of *Artemisia* species were between 9.79 µg GAE/mg and 15.38 µg GAE/mg. On the other hand, Lee *et al.* (2013) determined that myricetin amount was 1,086.55 mg/100g,

Table 1. Grown site of investigated *Artemisia* species

Species	Grown sites	Herbarium no.
A. armeniaca Lam.	Agri, Dogubeyazit, Bardakli village, 2565 m, N 39° 43.601, E 44° 03.501, 23.09.2007	1068
A. incana (L.) Druce	Bitlis, Adilcevaz, 1720 m, N 38° 47. 855, E 42° 43.000, 23.09.2007	1075
A. tournefortiana Reichb.	Van, Gurpinar, Hamurkesen village, 1953 m, N 38° 20.774, E 43° 37.377, 20.09.2007	1055
A. haussknechtii Boiss.	Hakkari, Kırıkdag village, 1624 m, N 37° 34.873, E 43° 54.148, 21.09.2007	1059
A. scoparia Waldst & Kit.	Ankara, Polatli road, 20 th km, 796 m, N 39° 42.876, E 32° 17.941, 10.09.2007	1030

Table 2. Fatty acid compositions (%) of investigated *Artemisia* species

SFA: Saturated fatty acids MUFA: Monounsaturated fatty acids PUFA: Polyunsaturated fatty acids USFA: Unsaturated fatty acids

Fatty acids (%)	*A. armeniaca*	*A. incana*	*A. tournefortiana*	*A. haussknechtii*	*A. scoparia*
Myristic acid	-	-	-	-	10.71±0.32
Palmitic acid	24.46±0.68	9.76±0.26	11.32±0.41	16.67±0.39	20.39±0.24
Margaric acid	-	4.37±0.54	-	-	-
Stearic acid	23.47±0.44	3.04±0.27	3.18±0.32	4.77±0.39	11.76±0.43
Arachidic acid	4.37±0,41	-	1.36±0.32	-	4.38±0.51
Behenic acid	4.02±0.46	-	1.04±0.19	10.47±0.21	3.01±0.23
Tricosylic acid	5.52±0.76	-	3.82±0.23	3.82±0.62	-
Lignoceric acid	3.37±0.33	-	1.62±0.36	4.11± 0.89	7.76±0.45
ΣSFA	65.21±0.51	17.17±0.35	22.34±0.3	39.84±0.49	58.01±0.36
Palmitoleic acid	3.37±0.33	3.08±0.55	6.46±0.32	8.61±0.32	4.53±0.27
Oleic acid	6.13±0.29	2.59±0.34	9.1±0.25	11.97±0.41	6.23±0.29
ΣMUFA	9.5±0.31	5.67±0.44	15.56±0.28	20.58±0.36	10.76±0.28
Linoleic acid	9.94±0.28	12.98±0.33	37.23±0.47	15.84±0.51	14.42±0.23
Eicosadienoic acid	4.77±0.38	23.41±0.32	12.24±0.28	6.68±0.33	1.81±0.27
Arachidonic acid	6.47±0.22	7.79±0.45	2.61±0.39	7.44±0.37	3.17±0.36
α-linolenic acid	4.42±0.33	22.21±0.43	1.34±0.32	-	-
γ-linolenic acid	-	2.25±0.42	3.83±0.39	-	2.07± 0.23
Stearidonic acid	-	9.11±0.52	5.86±0.49	9.72±0.27	9.83±0.41
ΣPUFA	25.6±0.3	77.75±0.41	63.11±0.39	39.68±0.37	31.13±0.3
ΣUSFA	35.1±0.3	83.42±0.42	78.67±0.33	60.1±0.32	42.06±0.29

Table 3. Vitamin and sterol contents of investigated *Artemisia* species

	Vitamins (µg/g)								Sterols (µg/g)		
Species	K2	K1	R-tocopherol	D2	D3	α-tocopherol	Retinol	Retinol acetate	Ergosterol	Stigmasterol	βsitosterol
A. armeniaca	0.67±0.12	2.72±0.28	0.16±0.09	0.32±0.11	-	23.12±1.21	0.14±0.02	0.13±0.06	164.75±2.34	1.81±0.13	26.5±1.23
A. incana	0.32±0.09	1.11±0.15	0.32±0.14	1.97±0.44	0.32±0.13	0.11±0.02	-	-	11.22±1.43	5.72±0.28	1.93±0.17
A. tournefortiana	1.21±0.16	2.68±0.23	2.51±0.16	5.17±0.38	0.87±0.23	-	-	-	3.07±0.76	16.76±1.57	7.53±0.67
A. haussknechtii	0.71±0.17	1.1±0.15	0.94±0.14	2.34±0.25	-	-	-	-	67.43±1.48	0.74±0.11	7.21±0.45
A. scoparia	-	1.43±0.22	-	0.32±0.18	0.56±0.18	-	-	1.71±0.15	-	2.11±0.24	0.62±0.13

Table 4. Flavonoid contents of studied *Artemisia* species

Flavonoids (µg/g)	*A. armeniaca*	*A. incana*	*A. tournefortiana*	*A.haussknechtii*	*A. scoparia*	Retention time (minute)
Catechin	11486.71±3.52	-	2684.87±3.42	-	-	1.63
Naringin	268.13±1.52	226.43±1.17	-	-	-	2.35
Rutin	6043.64±3.71	7259.43±3.49	-	5156.13±4.15	11416.11±3.43	3.12
Myricetin	17332.1±3.55	-	76.25±1.11	1861.44±1.77	111.79±2.34	4.24
Morin	1406.79±4.12	457.74±1.57	91.21±0.34	45.35± 0.65	256.78±1.21	5.09
Naringenin	191.18±1.22	15.32± 0.46	190.79±1.57	42.18±0.75	97.76±1.11	5.47
Quercetin	223.32±2.01	13.23±0.58	101.69±2.13	-	645.12±2.13	7.21
Kaempferol	36.56±0.35	-	21.74±0.65	-	262.19±1.38	12.39

quercetin amount was 30.90 mg/100g and kaempherol amount was 12.95 g/100 mg in the *Artemisia* studied. In addition, they measured rutin as 44.00 mg/100 g, resveratrol as 21.40 mg/100 g (Lee *et al.,* 2013). Nouria and Omar (2014) indicated that phenolic compounds, tannins and flavonoids were the major constituents of *Artemisia.* In contrast to these findings, some previous studies indicated that some *Artemisia* species had low or no flavonoid content (Wojdylo *et al.,* 2007; Li *et al.,* 2008).

Conclusions

The results of the present study showed that *Artemisia* species contained high saturated fatty acid compositions and linoleic acid, the major polyunsaturated fatty acid. Moreover, it was found that studied *Artemisia* species were rich in flavonoid constituents. Morin and naringenin were determined within all five species analysed. However, it was found that the sterol and vitamin contents of *Artemisia* species under the present study were low.

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