Journal of Drug Metabolism and Toxicology

Kurşat et al., J Drug Metab Toxicol 2014, 5:6
DOI: 10.4172/2157-7609.1000172

Research Article Open Access

Some Biological Contents and Radical Scavenging Activities of Five *Artemisia* L. Species Growing in Turkey

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Abstract

In the present study, fatty acid compositions, vitamin and sterol contents, flavonoid constituents and radical scavenging activity of extracts of the *Artemisia* species from Turkey (*A. absinthium* L., *A. vulgaris* L., *A. austriaca* Jacq., *A. verlotiorum* Lamotte and *A. caucasica* Willd.) are studied. Results of present study demonstrated that the main fatty acids in the extracts of *Artemisia* L. are determined as palmitic acid (C16:0; 7.1-25.9%), stearic acid (C18:0; 2.6-12.5%), palmitoleic acid (C16:1 n9; 1.3-13.1%), oleic acid (C18:1 n9; 1.5-20.3%), linoleic acid (C18:2 n6; 14.1-63.4%), docosadienoic acid (C22:2; 1.6-10.3%). Lipide-soluble vitamin contents of *Artemisia* L. species are found low concentrations. Also, it is determined that *A. absinthium* and *A. vulgaris* have rich in term of ergosterol content. Furthermore, it is showed that *Artemisia* L. species studied rich in flavonoid content apart from *A. vulgaris*. It is determined that quercetin and kaempferol contents of *Artemisia* L. species are low. In addition, it is observed that the studied *Artemisia* L. species posses strong DPPH radical scavenging activity.

Keywords: *Artemisia* L.; Fatty acids; Flavonoids; Radical scavenging activity; Vitamins; Sterols

Introduction

Artemisia L. (commonly wormwood or sagebrush) is the largest genus in the tribe *Anthemideae* (in the Asteraceae), with more than 300-500 species of herbs [1-3]. It is mainly distributed in the Northern hemisphere, particularly in West and Central Asia [1,4]. *Artemisia* is represented by 23 species in the Flora of Turkey [5]. According to our study the genus *Artemisia* is reported by 26 taxa in Turkey [6]. Many spices of *Artemisia* are known as aromatic plants and species of *Artemisia* are used for various purposes such as medicine, food, spices, and ornaments [7,8].

Several literatures indicated that the compounds obtained from different *Artemisia* species such as terpenoids, flavonoids, coumarins, and sterols showed a vast range of biological effects including cytotoxic, antimicrobial, anti-tumor, anti-diabetic and antioxidant activity [8-14]. Present study aimed to explore the fatty acids, vitamins and sterol contents, flavonoids and radical scavenging activities of five *Artemisia* species (*A. absinthium* L., *A. vulgaris* L., *A. austriaca* Jacq., *A. verlotiorum* Lamotte and *A. caucasica* Willd.) studied.

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The present study examined plant extracts of five *Artemisia* species: *A. absinthium* L., *A. vulgaris* L., *A. austriaca* Jacq., *A. verlotiorum* Lamotte and *A. caucasica* Willd. Sample plants were collected from their natural habitats and details about the seed materials are shown in Table 1.

Extraction of plant oils

Plant materials were finely ground in a mill and were then extracted with hexane/isopropanol (3:2 v/v) [15]. The lipid extracts were centrifuged at 10,000 g for 5 minutes and filtered, and the solvent was then removed on a rotary evaporator at 40°C. The extracted lipids were stored under -25°C until further analysis.

Fatty acids' analyses

Fatty acids in the lipid extracts were converted into methyl esters by means of 2% sulphuric acid (v/v) in methanol [16]. 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 Glass GC 10 software computer software. Chromatography was performed with a capillary column (25 m in length and 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.

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 S´anchez-Machado et al. with minor modifications [17]. The extracted lipids of seed material were dissolved in acetonitrile/methanol (75/25 v/v) and were injected 50 mL to HPLC instrument (Shimadzu, Kyota Japan). Column was used a Supelcosil TM LC18 (250 × 4.6 mm, 5 mm, Sigma, USA). 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 d-tocopherol, vitamin D, a-tocopherol, a-tocopherol acetate, 202 nm for phytosterols, 265 nm for vitamin K1. Identification of the individual vitamins and phytosterols were performed by frequent comparison with authentic external standard mixtures analyzed under the same conditions [18,19]. Class Vp 6.1

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Received October 01, 2014; **Accepted** November 06, 2014; **Published** November 10, 2014

Citation: Kurşat M, Yılmaz Ö, Emre I, Civelek Ş, Gökçe Z (2015) Some Biological Contents and Radical Scavenging Activities of Five Artemisia L. Species Growing in Turkey. J Drug Metab Toxicol 5: 172. doi:10.4172/2157-7609.1000172

software assisted at workup of the data. The results of analysis were expressed as µg/g for samples.

Flavonoid analysis and DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging capacity preparation of the extracts

2 g plant material was homogenized in 5 ml 80% methanol. Homogenates were centrifuged at 5000 rpm at +4ºC. After centrifugation, the supernatant was concentrated by reduced-pressure rotary evaporation. Each extract was re-suspended in dimethyl sulphoxide (DMSO) to produce a stock solution.

Chromatographic conditions for flavonoids

Chromatographic analysis was carried out using a PREVAIL C18 reversed-phase column (15×4.6 mm, 5 µm, USA); the mobile phase was methanol/water/acetonitrile (46/46/8, v/v/v) containing 1.0% acetic acid [18]. This mobile phase was filtered through a 0.45 µm membrane filter (Millipore), then de-aerated ultrasonically prior to use. 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 standart method. The results were expressed as µg/g tissue weight. All chromatographic operations were carried out at a temperature of 25°C.

Antioxidant assay by DPPH radical scavenging activity

The free radical scavenging effect of extracts was assessed by the decoloration of a methanolic solution of DPPH• according to the method of Liyana-Pathiranan et al. [20]. A solution of 25 mg/ L DPPH in methanol was prepared and 4.0 ml of this solution was mixed with 50, 100 and 250 µL of extract in DMSO. The reaction mixture was left in darkness at room temperature for 30 minutes. The absorbance of the mixture was measured spectrophotometrically at 517 nm. 1 µM quercetin was used as a reference.

The ability to scavenge DPPH radicals was calculated by the following equation: DPPH radical scavenging activity (%) = [(Abs control – Abs sample)]/(Abs control)] × 100 where Abs control is the absorbance of DPPH radical + methanol; Abs sample is the absorbance of DPPH radical + sample extract/standard.

Results and Discussion

Fatty acid compositions, vitamin and sterol contents of *artemisia* species

The constituent percentage composition of the fatty acids in five *Artemisia* is shown in Table 2. The main fatty acids in the extracts of *Artemisia* are determined as palmitic acid (C16:0; 7.1-25.9%), stearic acid (C18:0; 2.6-12.5%), palmitoleic acid (C16:1 n9; 1.3-13.1%), oleic acid (C18:1 n9; 1.5-20.3%), linoleic acid (C18:2 n6; 14.1-63.4%), docosadienoic acid (C22:2; 1.6-10.3%). Palmitic acid and stearic acid identified from all species but myristic acid (C14:0), arachidic acid (C20:0), behenic acid (C22:0) are absent or low amounts in the

Species	Locality	Herbarium no
Artemisia absinthium L.	Ağrı, Doğubeyazıt, Zor mountain, 2054 m	FUH 1063
A. vulgaris L.	Bitlis, Tatvan, Beşparmak village, 1718 m	FUH 1048
A. austriaca Jack.	Van, Gürpınar, Hamurkesen village, 1975 m	FUH 1054
A.verlotiorum Lamotte	Rize, Ardeşen-Çamlıhemşin road, 9 m	FUH 1105
A. caucasica Willd.	Niğde, Çamardı, Demirkazık village, 1560 m	FUH 1006

Table 1: Localities of studied Artemisia species.

Fatty acids (%)	*A. absinthium*	*A. vulgaris*	*A. austriaca*	*A. verlotiorum*	*A. caucasica*
14:0	0.4	0.9	-	-	-
16:0	12.3	13.4	22.1	25.9	7.1
18:0	9.6	3.8	12.5	5.3	2.6
20:0	0.5	2.1	-	-	-
22:0	0.4	2.5	-	3.8	-
23:0	16.0	1.4	-	-	-
24:0	1.9	0.7	6.7	2.3	-
ΣSFA	41.1	24.8	41.3	37.3	9.7
16:1 n7	1.1	0.4	-	-	-
16:1 n 9	3.5	5.7	6.6	13.1	1.3
18:1 n9	3.8	20.3	4.1	1.5	12.4
ΣMUFA	8.4	26.4	10.7	14.6	13.7
18:2 n-6	32.1	27.0	14.1	17.5	63.4
18:2 n6 T	1.0	1.2	-	-	1.8
20:2 n 6	0.6	2.1	2.7	-	-
22:2	3.3	2.0	10.3	4.7	1.6
18:3 n3	9.4	0.6	2.6	-	-
18:3 n6	4.2	3.1	-	-	9.2
18:4	-	12.6	18.2	26.2	-
ΣPUFA	50.6	48.6	47.9	48.4	76.0
ΣUSFA	59.3	75.0	58.6	63.0	89.7

Table 2: Fatty acid compositions of studied Artemisia species.

present study. Tricosanoic acid (C23:0) is determined 16.0% in the *A. absinthium* and lignorenic acid (C24:0) is determined 6.7% in the *A. verlotiorum*. It is only detected low saturated fatty acid composition in *A. caucasica* (9.7%).

Palmitoleic acid (C16:1 n9) and oleic acid (C:181 n9) are found the major monounsaturated fatty acid in the *Artemisia* species. Palmitoleic acid content is highest level in *A. verlotiorum* (13.1%) and lowest level in *A. caucasica* (1.3%) while oleic acid content is highest level in *A. vulgaris* (20.3%) and lowest level in *A. verlotiorum* (1.5%). Linoleic acid is major polyunsaturated fatty acid. *A. caucasica* has highest linoleic acid content (63.4%) while *A. austriaca* has lowest linoleic acid content (14.1%) among the studied five *Artemisia* species. α-linolenic acid and γ-linolenic acid are absent or low levels in present study. Also, eicosadienoic acid (C20: n6) is detected low percentage (0.6-2.7%) or absent. In addition, it is found that *A. vulgaris* (12.6%), *A. austriaca* (18.2%) and *A. verlotiorum* (26.2%) had high stearidonic acid content (C18:4). Fatty acid results of study from different genus of *Asteraceae* done by Orhan et al. suggested that most of the extracts seemed to be rich in in terms of saturated fatty acids [21]. They indicate that palmitic acid content of different species from *Asteraceae* is highest [21].

Sitosterol, campesterol and stigmasterol are the most common plant sterols in nature [22]. Stigmasterol (1-18.4 µg/g), beta-sitosterol (0.2-12.8 µg/g) are found in all five *Artemisia* species studied. Ergosterol is found highest ratio in *A. absinthium* (145.9 µg/g) and *A. vulgaris* (152.8 µg/g) (Table 3). Presence of sitosterol and ergosterol in *A. annua* demonstrated by Abid Ali Khan et al. [23]. Furthermore, present study showed that lipide-soluble vitamin contents of studied five *Artemisia* species were lowest values (Table 3). *A. absinthium* and *A. vulgaris* have highest α-tocopherol contents (25.1 µg/g and 5.4 µg/g, respectively) and *A. vulgaris* and *A. austriaca* have highest D2 vitamin contents (5.1 µg/g and 3.6 µg/g). Brisibe et al. found that vitamin A content of *A. annua* was found below 0.3 g/100 g while vitamin E was determined high levels in *A. annua* leaves (22.63 mg/kg) [24].

Flavonoid contents and radical scavenging activities of *Artemisia* species

Recent studies have focused on health functions of phenolics, including flavonoids from medicinal plants and many studies showed that the antioxidant activity of herbs and species caused by phenolic compounds [25-29]. Total nine flavonoids (rutin, myricetin, morin, quercetin, kaempferol, catechin, naringin, naringenin) are studied in this study (Table 4). *Artemisia* species generally known as rich antioxidant sources such as flavonoids, coumarins [24,30]. It is found that all studied species apart from *A. vulgaris* posses highest catechin content (3288.8-9877.6 µg/g) in the present study. It is found that kaempferol (3.8-52.5 µg/g) content of studied five *Artemisia* species are lowest. Also, *A. absinthium* and *A. verlotiorum* have higher quercetin constitiuent than other *Artemisia* species studied. In addition, *A. austriaca* and *A. caucasica* have highest rutin (1093.8-5997.7 µg/g, respectively) and naringenin (376.3-962.5 µg/g, respectively) constituents. Mino et al. reported that *Artemisia* species possess luteolin and kaempferol constituents [31]. Also, Djeridane et al. concluded that all studied plants including *Artemisia* species are rich in flavonoids [32]. Furthermore, several studies showed that different *Artemisia* species posses apigenin, luteolin, rutin, kaempferol, quercetin and naringenin constituents [33,34]. On the contrary, it is indicated that some *Artemisia* species didn't have or low flavonoid content [35,36].

The radical scavenging activities of *Artemisia* species are determined according to DPPH radical scavenging test (Table 4). These results showed that *Artemisia* species posses high radical scavenging activity. *A. verlotiorum* has highest radical scavenging activity while *A. absinthium* has weak antioxidant capacity. It is reported that antioxidative effectiveness of natural sources to be mostly due to phenolic compunds [7]. It is determined that several *Artemisia* species have strong antioxidant capacity and total phenolic contents [37-42]. showed the antioxidant activity of flavonoids from *A. vulgaris*; also Nam et al. observed antioxidant activities were increased by flavonoids in *Artemisia* extract [43,44].

Conclusion

Total saturated fatty acid compositions of studied *Artemisia* species are found high (apart from *A. caucasica*). Palmitic acid (C16:0) and stearic acid (C18:0) are found major saturated fatty acids. Also, it is determined myristic acid (C14:0), arachidic acid (C20:0), behenic acid (C22:0), tricosanoic acid (C23:0) and lignorenic acid (C24:0) in some *Artemisia* species. Linoleic acid (C18:2 n6) is determined as chief polyunsaturated fatty acid while linoleic acid (C18:3 n3) is found low amounts or absent. It is found that unsaturated faty acid compositions of *A. vulgaris* and *A. caucasica* were more than 70%. Vitamin contents

	Lipide-soluble vitamins (µg/g)								Sterols (µg/g)		
Species	**K2**	**K1**	**R-tocopherol**	**D2**	**D3**	**A-tocopherol**	**Retinol**	**Retinol acetate**	**Ergosterol**	**Stigmasterol**	**B-sitosterol**
A. absinthium	1.2	3.2	0.8	0.8	0.6	25.1	0.33	0.66	145.9	18.4	12.1
A. vulgaris	3.5	2.3	2.8	5.1	-	5.4	1.4	-	152.8	2.6	12.8
A. austriaca	-	-	0.1	3.6	0.2	0.2	0.7	-	-	1.0	5.6
A. verlotiorum	-	0.5	0.7	-	0.4	0.7	-	-	2.4	2.4	7.7
A. caucasica	-	2.1	0.2	0.7	0.4	0.7	-	-	1.5	1.5	0.2

Table 3: Lipid-soluble vitamin and sterol contents of studied Artemisia species.

	Flavonoids (µg/g)								Radical Scavenging Capacity (% inhibition)	
Species	**Rutin**	**Myricetin**	**Morin**	**Quercetin**	**Kaempferol**	**Catechin**	**Naringin**	**Naringenin**	25 µl	50 µl
A. absinthium	256.3	98.8	9368.1	240	36.3	9877.6	-	46.3	56.2%	67.1%
A. vulgaris	248.6	1367.5	8906.2	52.5	37	-	2720	89.5	95.2%	76.8%
A. austriaca	1093.8	-	1526.3	15	3.8	3288.8	-	376.3	68.2%	80.6%
A. verlotiorum	-	42.5	180	450	52.5	4332.5	-	17.5	96.1%	95.45%
A. caucasica	5997.5	67.5	18.8	-	31.3	3610	1253.8	962.5	71.8%	95.2%

Table 4: Flavonoid contents and DPPH radical scavenging capacity of studied Artemisia species.

of *Artemisia* species are low amounts. It is found that ergosterol content of *A. absinthium* and *A. vulgaris* are high amounts. Furthermore, it is showed that studied *Artemisia* species have high flavonoid content. In addition, it is observed that the studied *Artemisia* species posses strong DPPH radical scavenging activity. *A. verlotiorum* has the highest DPPH radical scavenging activity whilst *A. absinthium* has the lowest radical scavenging capacity among studied *Artemisia* species.

Acknowledgement

This study was supported by The Scientific and Technological Research Council of Turkey (TUBİTAK) with project number 106T559.

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