Journal of Reports in Pharmaceutical Sciences 2018, 7(3), 222-230

Chemical Composition and Antioxidant Activity of *Pistacia Atlantica Subsp. Kurdica* from Awraman

Seyed Mostafa Nachvak[a], Seyran Hosseini[a], Amir Nili-Ahmadabadi[b,c], Dara Dastan[b,d*], Mansour Rezaei[e]**

[a]Department of Nutrition, School of Health, Kermanshah University of Medical Sciences, Kermanshah, Iran
[b]Medicinal Plants and Natural Products Research Center, Hamadan University of Medical Sciences, Hamadan, Iran
[c]Department of Pharmacology and Toxicology, School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran
[d]Department of Pharmacognosy and Pharmaceutical Biotechnology, School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran
[e]Biostatistics and Epidemiology Department, Kermanshah University of Medical Science, Kermanshah, Iran

ARTICLE INFO

Article Type:
Research Article

Article History:
Received: 2018-03-09
Revised: 2018-04-25
Accepted: 2018-05-02
ePublished: 2018-05-29

***Keywords*:**
Pistacia Atlantica Subsp. *Kurdica*
Hulls
Essential Oil
Antioxidant
Phenolic Content
Flavonoid Content

A B S T R A C T

Pistacia atlantica subsp. *kurdica* is an important food source and a well-known medicinal plant in Zagros Mountains of Iran. The aim of this study was to evaluate phytochemical composition and biological activity of *P. atlantica* hulls. The phenolics profile of *P. atlantica* hulls extract was analyzed using the Reversed-phase high-pressure liquid chromatography equipped with photodiode array detector (RP-HPLC-PDA) and the chemical composition of essential oil was determined using the Gas chromatography–mass spectrometry (GC-MS) and Gas Chromatography – Flame Ionization Detector (GC-FID). The total phenolic and total flavonoid contents of hydro-alcoholic extract of *P. atlantica* hulls were measured by Folin–Ciocalteu and aluminium chloride colorimetric methods, respectively. The antioxidant activity of hydro-alcoholic extract was studied by using 2,2-diphenyl,1-picrylhydrazyl (DPPH) assay. Quantitative HPLC analysis indicated that ferulic acid, quercetin and naringenin are the main phenolic compounds in plant extract. In total, 55 compounds were identified and the main identified compounds of the essential oil were α-pinene and bornyl acetate. The hydro-alcoholic extract showed moderate antioxidant activity with IC_{50} =75 µg ml^{-1}. The total phenolic content of extract was 15.9 mg gallic acid equivalents/g dry weight, and the total flavonoid content of extract was 28 mg quercetin /g dry weight. The data of this study suggests that *P. atlantica* has potential for application as an antioxidant agent in pharmaceutical and food industries.

*Corresponding Author: Dara Dastan, E-mail: d.dastan@umsha.ac.ir
**CoCorresponding Author: Seyran Hosseini, E-mail: seyran.hosseini@gmail.com

Introduction

Pistacia atlantica subsp. *kurdica* belongs to the Ancardiaceae family originated from the Zagros Mountains of Iran. Oleoresin obtained from *P. atlantica* is used to make chewing gum, also hull and kernel oil are used as frying oil by natives. The unripe fruits of *P. atlantica* are used to make pickle and the ripe fruits are used as food by local people after mixing with other ingredients.

In addition to food consumption, *P. atlantica* has been employed in traditional medicine for the treatment of different diseases such as stomach disorders and throat infections [1]. According to literature it has been demonstrated that the *P. atlantica* and its chemical components produce a variety of pharmacological actions such as anticancer, antioxidant, anti-inflammatory and antimicrobial activities [2-4].

Free radicals or reactive oxygen species (ROS) may be formed through natural human physiological processes as well as from the environment. They may be the result of stress, alcohol, smoking, diet, exercise, inflammation, drugs or exposure to sunlight and air pollutants. Body can be removed free radicals generated in by its own natural antioxidant defense systems that include catalase, glutathione peroxidase, superoxide dismutase and etc that are not completely efficient, therefore dietary and natural antioxidants are required to reduce the effect of oxidative stress [5, 6].

The volatile composition of leaf, gum and kernel of *P. atlantica* subsp. *kurdica* were investigated but the volatile composition of *P. atlantica* subsp. *kurdica* hulls has not chemically been investigated so far [3, 7, 8]. The phytochemical study, HPLC profiles and biological activity of the *P. atlantica* subsp. *kurdica* from Awraman have not been investigated. Therefore, the present study aimed to investigate the chemical composition, HPLC profiles, antioxidant properties, total flavonoid and total phenolic content of the *P. atlantica* hulls from Avraman.

Materials and methods

Chemicals

2,2-diphenyl,1-picrylhydrazyl (DPPH), Butylated hydroxyl toluene (BHT), Folin–Ciocalteu reagent (FCR), quercetin, ferulic acid, caffeic acid, benzoic acid, naringenin, rutin, catechin, apigenin, epicatechin and gallic acid were obtained from Sigma Chemical Co. All other chemicals were of analytical grade.

Plant material

The hulls of *P. atlantica* were collected from wild population by randomized collection (Awraman, Iran), at an altitude of 1827 m. A voucher specimen (no. MPH-11854) is deposited in the Herbarium of the Research Institute of Forests and Range-land Research by Hossein Maroufi, Sanadaj, Iran.

Extraction of the essential oil

The volatile oil of hulls samples (80g) was isolated by hydro distillation for 3 h, using a Clevenger-type apparatus as previously reported. The volatile oil was dried by anhydrous sodium sulfate and, after filtration, stored at 5 °C until analyzed.

GC and GC-MS analyses

GC analysis was performed by using a Thermoquest gas chromatograph with a flame ionization detector (FID). The analysis was carried out using fused silica capillary DB-5 column (60 m × 0.25 mm; film thickness 0.25 μm). The carrier gas of GC was Nitrogen with flow rate of 1 mL min^{-1} as well as the temperatures of injector and detector were 250°C and 300°C, respectively. The oven temperature was programed from 50°C to 250°C at the rate of 5°C/min, and finally held isothermally for 10 min. GC-MS analysis was performed similarity GC column by using Thermoquest-Finnigan gas chromatograph coupled to a TRACE mass spectrometer. Ionization voltage was kept at 70 ev with Helium as a carrier gas. Also, interface and ion source temperatures

were kept 250°C and 200°C, respectively and mass range was scanned from 43 to 456 m/z [9].

Identification of compounds

Composition of the essential oil was identified by calculation of their retention indices in a DB-5 column under the same chromatographic conditions for *n*-alkanes (C_7-C_{24}). Compounds were identified by comparing their mass spectra with those of the library. As well as the GC retention indices of identified compounds were compared with those of reported in the literature and in finally the identified compounds were confirmed [10].

Preparation of extract

The hulls samples (100 g) were extracted by methanol/water (50/50) using maceration method. The solvent of extract was removed and concentrated by Rotary Evaporator apparatus to produce a dark gummy solid (12 g). The extract was kept in a vial in a cool and dark place for other stage of test.

Measurement of free radical scavenging activity (DPPH assay)

Antioxidant activity was studied by measuring the scavenging activity with the solution DPPH, using the method of Naseri et al. [11]. To prepare test solution one milliliter of extract was mixed with one milliliter of DPPH solution (500 µM). The absorbance of the blank (without sample) and positive control (BHT), was read at 517 nm after 1 h incubation without light on a Shimadzu UV-2100 spectrophotometer. Each sample assay was conclusion in triplicate and data is presented as a mean of the three values. The values were calculated as a percentage using the following formula [12].

% DPPH radical scavenging = (absorbance of blank-absorbance of sample) / (absorbance of blank) × 100

Determination of total phenolic content

The total phenolic content of the extract was measured by the Folin–Ciocalteu method [13]. 0. 2 mL of extract (10 g/L) were mixed to 20 mL distilled water and 1 mL of Folin–Ciocalteu reagent and after 4 min 3 mL of 7% (w/v) sodium carbonate was added to solution. After 60 min absorbance of solution was measured at 765 nm. The content of phenolic compounds in extracts was expressed as mg of gallic acid equivalent per g dry weight.

Determination of total flavonoid content

The total flavonoid content of extract was determined by the aluminium chloride colorimetric method [14]. 0.2 mL of extract (10 g/L) were mixed to 1.5 mL ethanol, 2.8 mL distilled water, 0.1 mL sodium acetate solution (1 M) and 0.1 mL of $AlCl_3$ solution (10%). After 60 min absorbance of solution was measured at 415 nm. The total flavonoid content was calculated from quercetin curve, and the result was expressed as mg quercetin equivalent per g dry weight.

Quantification of phenolic compounds by RP-HPLC-PDA

Two mg of extract was dissolved in 1 mL MeOH and was filtered through a 0.45µm/L filter. Seven different concentrations of phenolic standard (Sigma-Aldrich) were prepared in 1 mL MeOH ranging from 1 to 200 µg/mL. The HPLC column was a Spherisorb ODS-2 (5µm) reversed phase 4.6 mm × 250 mm andelution was carried out at a flow rate of 1.0 mL/min at 25ºC and detection at 270 nm with 20 µL sample injection volume (MeOH in H2O/Acetic acid (5–100% MeOH). Different parameters including UV spectra, retention times, and comparison with phenolic standard were used for the identification of phenolic compounds. The quantitative analysis was performed with external standardization by measurement of the peak areas using LabSolutions (Shimadzu) software.

Results

Hydrodistillation of *P. atlantica* hulls afforded a light yellow color volatile oil in 3.2% yield (w/w %) relative to dry weight of plant. In total, 55 compounds were identified representing 96.8% of the total essential oil. The identified compounds with retention indice and quantitative result are listed in Table 1, where all compounds are organized in order of their elution on the DB-5 column. The high content of *P. atlantica* subsp. *kurdica* essential oil identified by monoterpene hydrocarbons (57.3%) and the classification of compounds, based on their functional groups is summarized in Table 1.

Table 1. Composition of the essential oil of *Pistacia atlantica subsp. kurdica* hulls

Compounds	Percentage	RI[a]	RT[d]
Tricyclene	1.5	926	3.9
α-Pinene[c]	30.1	932	4.2
Camphene	5.7	953	4.4
2,4-Thujadien	1.0	957	4.5
Sabinene	0.2	969	4.8
β-Pinene	4.7	974	4.9
Myrcene	5.4	988	5.1
δ-2-Carene	0.1	1001	5.2
3-Carene	0.1	1011	5.5
α-Terpinene	0.2	1018	5.6
p-Cymene	0.6	1026	5.8
Limonene[c]	3.8	1031	5.9
β-Z-Ocimene	1.1	1040	6.0
β-E-Ocimene	0.5	1050	6.3
γ-Terpinene	0.3	1062	6.5
α-Terpinolene	2.1	1088	7.2
endo-Fenchol	0.3	1112	7.8
α-Campholenal	1.3	1125	8.1
trans-Pinocarveol	1.9	1139	8.5
Pinocarvone	1.2	1162	9.0
Borneol	1.2	1165	9.2
p-Mentha-1,5 dien-8-ol	1.0	1172	9.3
α-Terpineol	4.1	1189	9.8
Myrtenal	1.1	1193	9.9
Verbenone	0.6	1204	10.3
Bornyl acetate	8.5	1285	12.2
3Z-Hexenyl tiglate	0.1	1322	13.1
3Z-Hexenyl hexenoate	0.0	1333	13.4
α-Cubebene	0.1	1351	14.5
α-Copaene	0.1	1376	14.6
n-Tetradecane	t[b]	1399	15.0

Continue of Table 1. Composition of the essential oil of *Pistacia atlantica subsp. kurdica* hulls

Compounds	Percentage	RI[a]	RT[d]
trans-Caryophyllene	1.9	1418	15.6
β-Gurjunene	0.1	1432	16.1
α-Humulene	0.5	1452	16.4
γ-Muurolene	0.1	1477	16.6
ar-Curcumene	0.5	1480	17.0
Germacrene D	2.7	1480	17.1
Bicyclogermacrene	1.9	1494	17.5
Cuparene	0.1	1502	17.7
γ-Cadinene	0.3	1513	17.9
δ-Cadinene	0.9	1524	18.1
α-Cadinene	0.1	1538	18.4
α-Calacorene	0.3	1548	18.6
Elemol	0.1	1547	18.7
E-Nerolidol	0.2	1564	19.0
3Z-Hexenyl benzoate	1.5	1570	19.2
Spathulenol	5.3	1576	19.4
Humulene epoxide	0.1	1606	20.1
1,10-di-epi-Cubenol	0.2	1618	20.6
γ-Eudesmol	0.1	1630	20.7
β-Eudesmol	0.5	1649	21.1
α-Cadinol	0.5	1653	21.2
Benzyl Benzoate	0.1	1762	23.5
Methyl hexadecanoate	0.1	1927	24.7
Phytol	0.1	1949	25.1
Monoterpene hydrocarbons	57.3		
Oxygenated monoterpenes	21.2		
Sesquiterpene hydrocarbons	9.6		
Oxygenated sesquiterpenes	8.5		
Others	0.2		
Total	**96.8**		

[a]RI, Retention indices relative to C7 – C24 n-alkanes on the DB-5 column. [b]t, Trace < 0.1%.
[c] The identification was also confirmed by co-injection with an authentic samples. [d] Retention time of compounds.

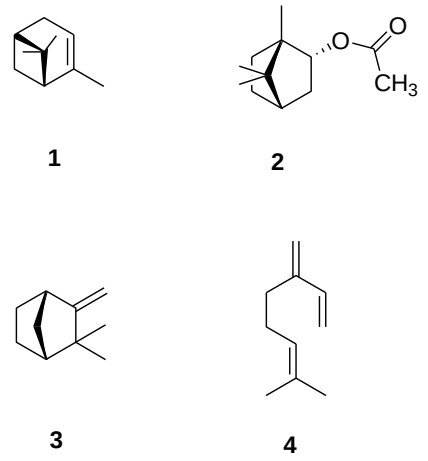

Fig. 1. The structure of P. *atlantica subsp. Kurdica* essential oil compounds. α-pinene (1), bornyl acetate (2), camphene (3), myrcene (4).

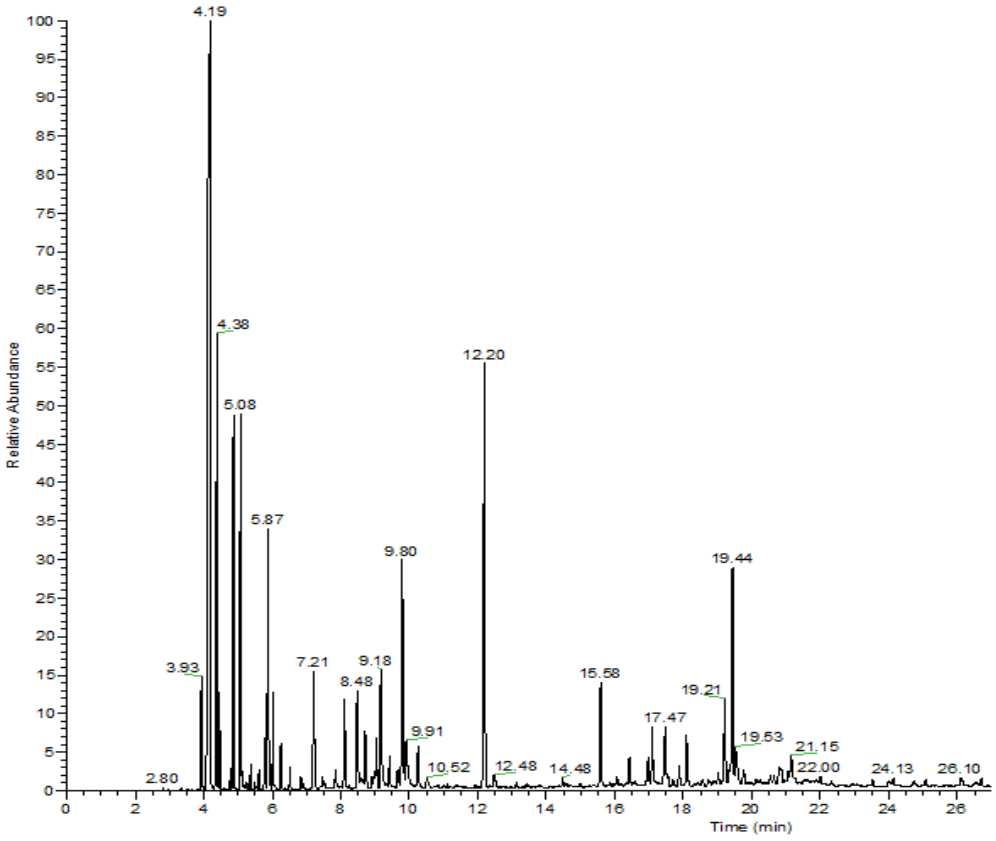

Fig. 2. The GC/Mass chromatogram of P. *atlantica subsp. Kurdica* essential oil.

The hydro-alcoholic extract showed moderate antioxidant activity with IC_{50} =75 µg ml^{-1}. The total phenolic content of extract was 15.9 mg gallic acid equivalents/g extract, and the total flavonoid content of extract was 28 mg quercetin /g extract. HPLC analysis of *P. atlantica* subsp. *kurdica* hulls showed ferulic acid was the most abundant

phenolic component in hydro-alcoholic extract followed by quercetin, naringenin, catechin, caffeic acid, apigenin, gallic acid, benzoic acid, rutin and epicatechin (from 120.2 to 7.2 µg/g dry weight) in Table 2.

Table 2. Quantitative results for determination of phenolic components in the *Pistacia atlantica subsp. kurdica* hulls

Phenolic compounds	Phenolic content (µg/g DW)	Retention time	λmax
Ferulic acid	120.2 ± 1.4	3.11	320
Gallic acid	29.4 ± 0.4	6.02	270
Caffeic acid	36.1 ± 0.5	22.34	295
Benzoic acid	24.6 ± 0.5	28.65	250
Naringenin	48.4 ± 0.8	30.20	285
Rutin	12.3 ± 0.6	33.04	360
Quercetin	68.1 ± 0.7	41.63	370
Catechin	41.2 ± 1.1	44.46	276
Apigenin	32.1 ± 0.2	52.62	336
Epicatechin	7.2 ± 0.4	54.12	276

Results are mean of three replicates with standard errors (Means ± S.E, n= 3), p< 0.05.
DW: dry weight

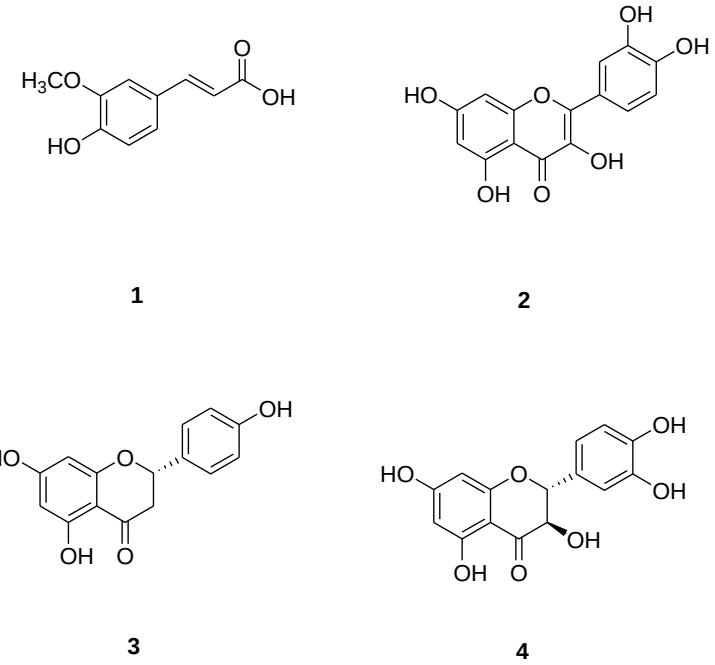

Fig. 3. The structure of P. *atlantica subsp. Kurdica* phenolic compounds. ferulic acid (1), quercetin (2), naringenin (3), catechin (4).

Discussion

The main identified compounds of the essential oil were α-pinene (30.31%), bornyl acetate (8.5%), camphene (5.7%), myrcene (5.4%), spathulenol (5.3%), β-pinene (4.7%), α-terpineol (4.1%), and limonene (3.8%), respectively (Fig. 1 and 2). In the study by Sharifi the α-pinene was the major

constituent of *P. atlantica subsp. Kurdica* gums. Similarity of results the major compounds in *P. atlantica subsp. Kurdica* hulls was α-pinene but the other major and minor essential oil constituent of hulls were different from leaf, gum and kernel of *P. atlantica subsp. Kurdica* [3].

The effect of antioxidant activity on DPPH radical scavenging is thought to be due to their hydrogen donating ability or radical scavenging activity. When a substance that can donate a hydrogen atom is mixed with solution of DPPH, this then gives rise to the reduced form with the loss of its violet color [15]. The DPPH scavenging abilities of the extract (IC_{50} =75 μg ml^{-1}) was lower than that of the synthetic antioxidant tert-butylatedhydroxytoluene ($IC_{50\ BHT}$ =26 μg ml^{-1}). The total phenolic content of *P. atlantica* extract, calculated from the calibration curve (R^2 = 0.9998), was 15.9 mg gallic acid equivalents/g dry weight, and the total flavonoid content (R^2 = 0.9999) was expressed as 1.8 mg quercetin /g dry weight. According to literature the antioxidant activity of a plant extract is correlated to its phenolic content [16].

Phenolic compounds as the major secondary metabolite of plants including phenolic acid and flavonoids have been shown to have important role in the treatment of different disorders such as Parkinson's disease, Alzheimer's, atherosclerosis, tumors and diabetes disease [17]. The quantification analysis of phenolic compounds of the hydro-alcoholic extract of *P. atlantica* subsp. *kurdica* hulls from Awraman were carried out using a RP-HPLC-PAD and ferulic acid was the most abundant phenolic component (Fig. 2). In the previous study by Hatmania et.al on *P. atlantica* subsp. *kurdica* hulls collected from Ghassemlu, Bane, Ghalajeh, Kolkol and Ghalarang the abundant phenolic component was syringic acid, sinapic acid, p-coumaric acid, syringic acid and gallic acid respectively [1]. According to results of our study and literature the amount of ferulic acid in *P. atlantica* subsp. *kurdica* hulls from Awraman, Ghassemlu, Bane, Ghalajeh, Kolkol and Ghalarang was 120.2, 48.6, 294.8, trace, 45.4 and 45.5 μg/g dry weight respectively [1].

Antioxidant activity, total phenolic content and total flavonoid content of *P. atlantica subsp. Kurdica* suggests that the essential oil and hydro-alcoholic extract of *P. atlantica* hulls has great potential for application as a natural antioxidant agent.

Conclusions

The data of this study suggests that the essential oil and hydro-alcoholic extract of *P. atlantica* hulls has potential for application as an antioxidant agent in pharmaceutical and food industries. The *P. atlantica* as an edible plant recommended as a suitable source for phenolic and flavonoids compounds in people nutrition. Also the data of this study can be used for further study on the taxonomy of *Pistacia* genus.

Conflict of interest

Authors certify that there is no actual or potential conflict of interest in relation to this article.

Acknowledgement

The authors gratefully acknowledge the Research Council of Kermanshah University of Medical Sciences (Grant Number: 96166) for the financial support. This work was performed in partial fulfillment of the requirements for MSc of Seyran Hosseini, in faculty of Nutrition, Kermanshah University of Medical Sciences, Kermanshah, Iran.

References

[1] Hatamnia AA, Abbaspour N, Darvishzadeh R. Antioxidant activity and phenolic profile of different parts of Bene (*Pistacia atlantica subsp. kurdica*) fruits. Food Chem. 2014;145:306-311.

[2] Heidarian E, Jafari-Dehkordi E, Valipour P, Ghatreh-Samani K, Ashrafi-Eshkaftaki L. Nephroprotective and anti-inflammatory effects of *Pistacia atlantica* leaf hydroethanolic extract against gentamicin-induced nephrotoxicity in rats. J Diet Suppl. 2017;14:489-502.

[3] Bozorgi M, Memariani Z, Mobli M, Salehi Surmaghi M, Shams-Ardekani M, Rahimi R. Five Pistacia species (*P. vera, P. atlantica, P. terebinthus, P. khinjuk, and P. lentiscus*): a review of their traditional uses, phytochemistry, and pharmacology. Sci World J. 2013;15:1-32.

[4] Rigane G, Ghazghazi H, Aouadhi C, Ben Salem R, Nasr Z. Phenolic content, antioxidant capacity and antimicrobial activity of leaf extracts from *Pistacia atlantica*. Natural Product Research. 2017;31:696-699.

[5] Badiee MS, Nili-Ahmadabadi H, Zeinvand-Lorestani H, Nili-Ahmadabadi A. Green tea consumption improves the therapeutic efficacy of deferoxamine on iron overload in patients with [Beta]-thalassemia major: a randomized clinical study. Biological Forum. 2015;7:383-388.

[6] Mumivand H, Rustaii A-R, Jahanbin K, Dastan D. Essential oil composition of *Pulicaria dysenterica* (L.) Bernh from Iran. J Essent Oil Bear Pl. 2010;13:717-720.

[7] Naseri NG, Ashnagar A, Naghdi N. Isolation and Identification of the Major Chemical Compounds Found in the Oleoresin Obtained from the *Pistachia atlantica* Tree (PersianTurpentine Tree, Desf. Subsp. Kurdica) Grown in Ilam Province of Iran. Asian J Chem. 2006;18:1121.

[8] Rahimi AR, Hadian J, Azizi M, Abdosi V, Larijani K. Quantity and Quality of Essential Oil of *Pistacia atlantica* Subsp. Kurdica in Response to Gradual Harvest of Oleoresin. J Essent Oil Bear Pl. 2016;19:616-623.

[9] Mohammadi M, Yousefi M, Habibi Z, Dastan D. Chemical composition and antioxidant activity of the essential oil of aerial parts of *Petasites albus* from Iran: a good natural source of euparin. Nat Prod Res. 2012;26:291-297.

[10] Shibamoto T. Retention indices in essential oil analysis in capillary Gas Chromatography, 259-275 p. Essential oil analysis Sandra P, Bicchic A (Eds) Alfred Heuthig-Verlag New York. 1987.

[11] Naseri M, Monsef-Esfehani H, Saeidnia S, Dastan D, Gohari A. Antioxidative coumarins from the roots of *Ferulago subvelutina*. Asian J Chem. 2013;25:1875-1879.

[12] Xiong L, Peng C, Zhou Q-M, Wan F, Xie X-F, Guo L, et al. Chemical composition and antibacterial activity of essential oils from different parts of *Leonurus japonicus* Houtt. Molecules. 2013;18:963-973.

[13] Kaur C, Kapoor HC. International Journal of Food Science and Technology. Int J Food Sci Technol. 2002;37:153-161.

[14] Chang C-C, Yang M-H, Wen H-M, Chern J-C. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002;10:124-129.

[15] Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol. 2004;26:211-219.

[16] Abdali E, Javadi S, Akhgari M, Hosseini S, Dastan D. Chemical composition and biological properties of *Satureja avromanica* Maroofi. J Food Sci Technol. 2017;54:727-734.

[17] Caleja C, Ribeiro A, Filomena Barreiro M, CFR Ferreira I. Phenolic Compounds as Nutraceuticals or Functional Food Ingredients. Curr Pharm Des. 2017;23:2787-806.

