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Original Article

Investigation of Phytochemical and Antioxidant Capacity of Fennel (*Foeniculum vulgare* Mill.) Against Gout

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Abstract

Fennel, *Foeniculum vulgare* Mill. (Apiaceae) is an aromatic plant, with medicinal and culinary applications, widely naturalized worldwide, especially near the see coasts and riversides. Fennel has long been used in Iran as a traditional remedy against gout. The objective of our study was to uncover the scientific basis of this traditional gout therapy. Different plant parts were extracted by methanol and used in phytochemical assessment and examined for possible inhibitory effects on xanthine oxidase -the main enzyme responsible for uric acid accumulation in blood. FRAP and *β*-carotene bleaching assays, total anthocyanin, carotenoids, soluble sugars, phenolics and flavonoids content were measured spectrophotometrically, while caffeic acid, chlorogenic acid and quercitin contents were measured by HPLC. The experiments were performed using a 3-stage nested statistical design with three biological replications. Results showed that the flower extract exhibited the most xanthine oxidase inhibitory effect (80% of Allopurinol), the highest amounts of total phenol, flavonoid and cafeic acid (53.55, 7.71 and 0.049 mg gdw^{-1}, respectively), as well as significant antioxidant activity in scavenging free radicals. These results suggest that flower extract of fennel is a natural source of valuable compounds against xanthine oxidase activity, with potential therapeutic applications in human gout treatment.

Keywords: *Foeniculum vulgare*, Xanthine Oxidase, Phenolics, Flavonoids, Gout

Introduction

Elevated levels of uric acid in blood often lead to hyperuricemia related diseases such as gout. In human, gout can be developed by elevated production of uric acid via *de novo* biosynthesis, or breakdown of nucleic acids, and ablated elimination of uric acid via kidney [1]. In the first two mechanisms, conversion of hypoxanthine to xanthine and subsequently to uric acid is catalyzed by an enzyme named xanthine oxidase [2]. Accordingly, in a therapeutic approach against gout, an inhibitor of xanthine oxidase named Allopurino, as an approved synthetic drug, is widely prescribed; although it has many side effects [1].

In addition to uric acid, in the above indicated reactions catalyzed by xanthine oxidase, H_2O_2 and $O_2°$ are produced, which in turn can induce several cellular modifications, including DNA damage, protein carbonylation and lipid peroxidation, some of which may lead to inflammatory reactions [3, 4]. Application of antioxidants may help patients suffering from gout to tolerate joint inflammation caused by accumulation of hydrogen peroxide and super-oxide molecules. Due to anticipated side effects of synthetic drugs [5], demand for remedies obtained from natural resources, such as medicinal plants, that prevent accumulation of uric acid and oxidative damage is increasing.

Foeniculum vulgare Mill. commonly known as fennel, belongs to the Apiaceae family with aromatic odor, medicinal and culinary applications, and is widely cultivated throughout the temperate and tropical regions of the world, including Iran (known as Razianeh) [6]. Fennel extracts exhibits carminative, flavoring, antioxidant, antibacterial,

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fungicidal, diuretic, lactation, stimulant and mosquito-repellent properties [7,8].

In ancient Greece, some prescriptions against human gout contained fennel extracts [11]. Likewise, in traditional medicine of India [12] and Iran [6] fennel was supposed to mitigate gout and act as a diuretic agent by increasing the flow of urine. Since, joint inflammation observed in gout is mainly caused by accumulation of mono sodium urate crystals leading to inflammatory reactions, we hypothesized that fennel extracts, in addition to anti-inflammatory effects, might exhibit a direct inhibitory activity on production of uric acid. Therefore, Xanthine oxidase-inhibition potential and antioxidant properties of fennel extracts collected from different plant organs during various growth stages were examined.

Material and Methods

Plant Collection and Extraction

Wild type *Foeniculum vulgare* plants, were cultivated in Khaf, Northeast of Iran (34º North latitude to 60º East longitude). The plant identity was confirmed as *F. vulgare* with the herbarium code of 38425; by the Botanical Research Institute of Mashhad University, Iran. Different parts of the plant, collected during various stages of plant growth were separated and dried (Table 1). For extraction, one gram of each sample was soaked in methanol for 48 h at room temperature and kept at 4 ˚C for further analysis.

Biochemical Measurements

Xanthine oxidase activity was assayed according to the procedure of Noro *et al.* [13] as previously described [14]. Antioxidant activity of the extracts was assessed by FRAP, using the Benzene and Strain method [15] as well as by *β*-carotene bleaching assay [14]. Total anthocyanin and carotenoid contents were determined according to Mita *et al.* [16] and Lichtenthaler [17], respectively. Soluble sugar content was measured by phenol-sulfuric acid method, as described by Kochert, [18]. Total phenolic content was measure by the Folin–Ciocalteu method [18] and expressed as gallic acid equivalents. Total flavonoids were quantified using aluminum chloride reagent ($AlCl_3$) according to Chang *et al.* [20] and expressed as quercetin equivalent.

Caffeic acid, chlorogenic acid, quercetin and gallic acid contents were measured by High-Performance Liquid Chromatography (HPLC), as described by Owen *et al.* [21]. The HPLC was performed by a Hitachi model, UV/VIS detector, C18 column (Lichrospher 100 reversed phase, 250*4 mm; 5 µm). Mobile phases included water, methanol and acetic acid with an 80:18:2 ratio for caffeic acid, chlorogenic acid and gallic acid; and a 60:39:1 ratio for quercetin with a 1 ml/min flow rate. The UV detector was operated at 330 nm for caffeic acid and chlorogenic acid, 280 nm for gallic acid and 360 nm for quercetin, respectively. 100 µl of samples were injected into equipment and 20 µl of each were loaded.

Statistical Analyses

All experiments were conducted at least in triplicates and the results were reported as mean ± standard error (SE). The experiments were performed according to a 3-stage nested design and statistically significant differences at the %5 confidence level were determined using the LSD method. The correlation index was used to confirm the effect of parameters on Xanthine oxidase inhibition, using SAS (9.2) and Minitab (16.1.1) software.

Result and Discussion

The Inhibitory Effect of Plant Extract on Xanthine Oxidase Activity

In the traditional Uighur formula known as Karapxa Decoction [22] as well as Iranian traditional medicine, *F. vulgare* was used to treat gouty patients [6]. In order to study the scientific basis for these traditional applications, we extracted various organs of the plant at different growth stages (table 1) and measured there *in vitro* anti-xanthine oxidase activity. Data depicted in Figure 1 suggest that the lowest percentage of xanthine oxidase inhibition was found in stem of fruiting stage while the maximum capacity of inhibition was observed in flowers (82± 2.6%).

Determination of Antioxidant Capacity of Fennel Extracts

Since hydrogen peroxide and super-oxide molecules are by-products of xanthine oxidase activity, we reasoned that fennel extracts might reduce the side effects of gout through scavenging reactive oxygen species. Therefore, total antioxidant activities of fennel extracts during

various plant growth stages were measured by FRAP and β-carotene bleaching assays. Data presented in Table 2 indicate that all analyzed samples demonstrated significant antioxidant activity. These data suggest that traditional application of fennel extracts against gout might be related to the plant antioxidant potential.

Biochemical Basis of Fennel Antioxidant Activity

In order to determine potential compounds that might directly or indirectly scavenge hydrogen peroxide and super-oxide molecules, produced by xanthine oxidase, the amount of several compounds in various plant extracts were identified and presented in Table 2. Total phenolic content was higher in flowers (53.55 ± 1.41 mg GA.$g^{-1}dw^{-1}$) and leaves of the flowering stage (30.37 ± 0.35), as compared to other organs. Phenolic compounds which are commonly found in both edible and non-edible plants have been reported to exhibit multiple biological effects, including antioxidant activity [23-25].

Among the samples, flowers (7.71±0.14 mg Q.$g^{-1}dw^{-1}$) and leaves of the vegetative state (5.55± 0.26) showed the highest amounts of flavonoids (Table 2). Many of the pharmacological effects of flavonoids are linked to their known biological functions as antioxidants, due to free-radical scavenging and metal chelating [24].

Total anthocyanin content represented a dynamic change in different stages of plant growth and development. The highest amount of anthocyanin was detected in leaves of the flowering stage (7.56± 0.25 mg/gdw) while the lowest amount was detected in stem and root of the vegetative stage. It has been shown that anthocyanins are strong antioxidants with free radical scavenging properties, attributed to the phenolic hydroxyl groups present in their structures, while different hydroxylation and glycosylation may modulate their antioxidative properties [26].

The highest amounts of carotenoids were observed in leaves. Beta-carotene and other carotenoids are believed to provide antioxidant protection to lipid rich tissues, similar to vitamin E [27]. Carotenoids have also been linked with improvement of human immune system and decreased risk of degenerative diseases, such as cancer, cardiovascular disease, age-related macular degeneration and cataract formation [28].

The highest amount of soluble sugars was observed in the leaves of flowering stage and roots of fruiting stage and the lowest amount of that was in stems of vegetative stage. Many beneficial health properties of agaro-oligosaccharides have been attributed to their anti-oxidative activities in scavenging free hydroxyl and superoxide anion radicals, and inhibiting lipid peroxidation [29].

The Content of Caffeic Acid, Chlorogenic acid, Quercetin and Gallic Acid

To determinate which one of the phenolic compounds is effective in antioxidant activity and xanthine oxidase inhibition, caffeic acid, chlorogenic acid and gallic acid contents and quercetin, as an important flavonoid were assayed by HPLC (Table 3). The results showed that the caffeic acid was the highest in flowers (0.05 mg $g^{-1}dw^{-1}$) followed by leaves of flowering stage (0.02 mg. $g^{-1}dw^{-1}$). A similar trend was observed in total phenolic compounds (Table 2). The chlorogenic acid was the highest in leaves of vegetative stage (5.98 mg $g^{-1}dw^{-}1$) and flowers (2.85 mg $g^{-1}dw^{-1}$) with any specific order. The quercetin content was very low and negligible.

A correlation analysis showed a significant linear correlation between xanthine oxidase inhibition and phenolic/flavonoid contents of the extracts (Table 4). Among the phenolic compounds, caffeic acid which was higher in flowers was correlated with xanthine oxidase inhibition (Table 4). The HPLC results showed that pure caffeic acid had a low concentration in the extracts (Table 4) suggesting that most caffeic acid molecules were converted to its derivatives. Accordingly, Faudale *et al*. [30] reported that in fennel the amount of caffeic acid derivatives are high. Several experimental models showed that flavonoids and other phenolic compounds are considered as antioxidants not only because of their ability as free radical scavengers, but also because of their ability to directly inhibit Xanthine oxidase [31]. It is possible that the inhibitory effect of many phenolic compounds on xanthine oxidase activity is caused by their similar structures or by non-competitive inhibition.

Studies have shown that xanthine oxidase inhibitors might also be useful for successful treatment of hepatic disease, which is caused by the generation of uric acid and superoxide anion radicals [32]. Xanthine oxidase-derived superoxide anion has been linked to post-ischaemic tissue injury and edema [33]. Therefore, control of hydrogen peroxide and superoxide anions is very important

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Table 1 Plant growth stages and date of their collections.

Sampling stage	Phenological stage	Date of collection
1	sowing	24/3/2012
2	Vegetative stage	22/5/2012
3	Flowering stage	24/6/2012
4	Fruiting stage	10/8/2012

Table 2 Total phenol, flavonoid, anthocyanin, carotenoid and soluble sugar content of Foeniculum vulgare Mill. and antioxidant activity in the presence of plant extract from different stages and several organs.

	Vegetative stage			Flowering stage				Fruiting stage		
	Leaf	Stem	Root	Leaf	Stem	Root	Flower	Stem	Root	Fruit
Phenol content[1]	26.59±0.19	3.60±0.06	1.73±0.01	30.73±0.35	6.04± 0.30	5.91±0.04	53.55±1.41	1.15±0.01	2.81±0.17	24.18±1.71
Flavonoid content[2]	5.55±0.26	0.89±0.09	0.24±0.03	4.84±0.15	0.77±0.08	0.37±0.01	7.71±0.14	1.40± 0.02	0.12±0.01	2.29±0.04
Anthocyanin content[3]	2.65±0.44	1.52±0.19	1.49±0.24	7.57±0.26	4.85±0.02	5.51±0.13	4.43±0.12	5.68± 0.10	4.24±0.07	5.50±0.22
Carotenoid content[3]	5.15±0.21	0.59±0.03	0.025±0.00	6.67±0.29	0.98±0.17	0.22±0.07	1.71± 0.01	2.26± 0.16	0.67±0.03	1.80±0.03
Soluble Sugar content[3]	19.53±0.12	8.96±0.31	12.91±0.41	23.09±0.31	14.56±0.15	15.89±0.15	11.85±0.34	11.27±0.22	23.42±0.15	17.37±0.21
Antioxidant activity (FRAP)	0.03±0.00	0.02±0.00	0.01± 0.00	0.04± 0.00	0.03± 0.00	0.02±0.00	0.04±0.00	0.04± 0.00	0.02±0.00	0.04±0.01
Antioxidant activity (BCB)	69.39±0.16	83.99±1.69	77.26±1.21	79.92±0.27	96.05±1.52	82.42±0.83	92.96±1.56	86.67±1.32	93.78±2.63	83.68±0.68

[1](mg GA. $g^{-1}dw^{-1}$); [2](mg Q.$g^{-1}dw^{-1}$); [3](mg.$g^{-1}dw^{-1}$)

Table 3 Caffeic acid, chlorogenic acid, quercetin and gallic acid contents of Foeniculum vulgare Mill. from different stages and several organs.

Samples	Vegetative stage			Flowering stage				Fruiting stage		
Test	Leaf	Stem	Root	Leaf	Stem	Root	Flower	Stem	Root	Fruit
Caffeic acid content (mg. g-$^{1}dw^{-1}$)	0.02	0.02	0	0.03	0.02	0	0.05	0.02	0.01	0.02
Chlorogenic acid content (mg.$g^{-1}dw^{-1}$)	5.98	0.22	0.07	1.71	0.33	0.59	2.85	0.35	0.08	0.98
Quercetin content (mg.$g^{-1}dw^{-1}$)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gallic acid content (mg.$g^{-1}dw^{-1}$)	0.01	0.00	0.00	0.00	0.01	0.01	0.00	0.01	0.01	0.01

Table 4 Correlation between the inhibition percent of xanthine oxidase and several factors of Foeniculum vulgare Mill. samples.

	Phenol	Flavonoid	Anthocyanin	Carotenoid	Soluble Sugar	Caffeic acid	Chlorogenic acid	Quercetin	FRAP	BCB
Antioxidant activity (FRAP)	0.630**	0.659**	0.637**	0.639**	0.150	0.715**	0.369	0.640**	-	-
Antioxidant activity (BCB)	-0.046	-0.173	0.259	-0.458	-0.137	0.268	-0.517**	-0.355	-	-
Xanthine Oxidase inhibition	0.826**	0.676**	0.276	0.252	0.300	0.554**	0.312	0.348	0.280	0.036

** The level of significance is less than 1% ($p< 0.01$).

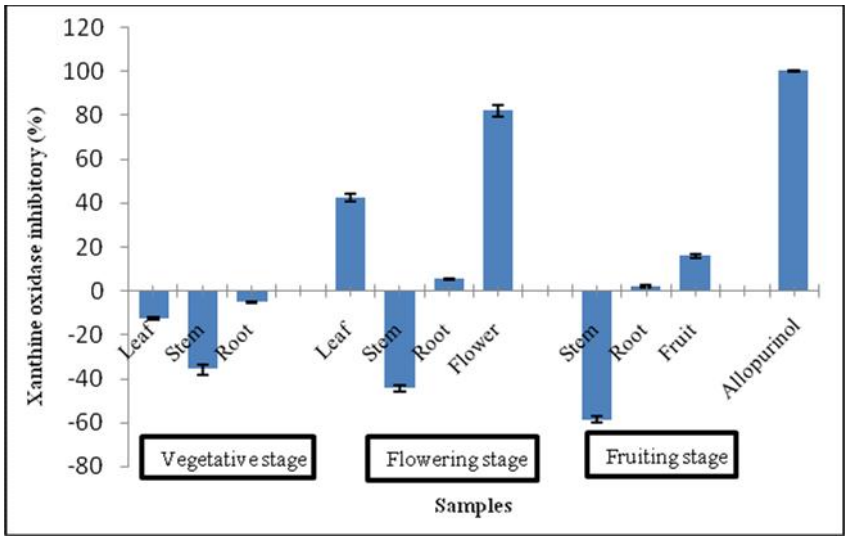

Fig. 1 Xanthine oxidase inhibitory percent of the Foeniculum vulgare Mill. extracts in different stages and several organs.

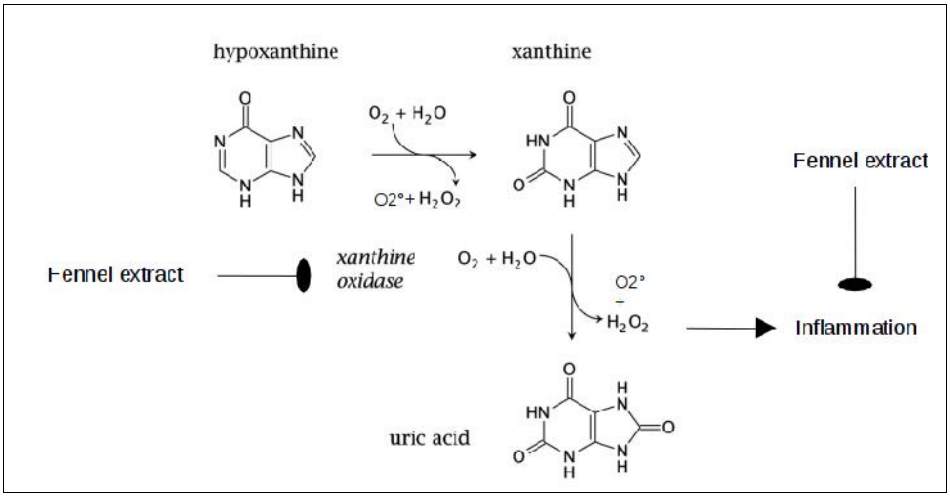

Fig. 2 Enzymatic conversion of hypoxanthine to xanthine and subsequently to uric acid is catalyzed by xanthine oxidase. Fennel extract may perform anti gout activity via direct inhibition of xanthine oxidase and indirect anti inflammatory effects.

Therefore, we can conclude that fennel extract contains various phenolic and flavonoid compounds, which perform divergent types of antioxidant activities, detected by different methods, some of which can reduce the side effects of xanthine oxidase activity. Altogether, data presented here suggest that fennel extracts may perform anti gout activity via two distinct pathways (Fig. 2). First of all, It may contain compounds with similar structures to xanthine oxidase natural substrate and, thus exhibit competitive inhibitory effects on the enzyme activity leading to reduced uric acid; and secondly, antioxidative compounds present in the plant extract may reduce inflammatory effects of uric acid and H_2O_2 accumulations.

Conclusions

In Iranian traditional medicine, fennel (*F. vulgare*) has been prescribed for treating gout disease. Here, we tried to uncover the scientific basis of this traditional gout therapy. Plant extracts obtained from various organs during different phenological stages were examined on (i) direct inhibition of xanthine oxidase activity and (ii) scavenging of free radicals resulted by xanthine oxidase activity. The results showed that the flower extract of fennel, which exhibited the highest inhibitory effect on xanthine oxidase activity contained the highest amounts of various phenolic/flavonoid compounds. In the phenolic and flavonoid compounds caffeic

acid and quercetin, despite their relatively low contents, showed a good correlation with xanthine oxidase inhibitory effects. On the other hand, the chlorogenic acid content did not show significant correlation with xanthine oxidase inhibition. In addition, we showed that the flower extracts of fennel contain considerable amounts of antioxidants with potential effects on scavenging of free radicals produced by the xanthine oxidase enzyme. Thus, our data justifies traditional application of fennel for gout treatment. Further, in depth *in vivo* analysis is needed for confirming these data and for suggesting the proper dose of the extract to combat gout in human.

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References

1. Poom SH, Hall HA, Zimmermann B. Approach to the treatment of hyperuricemia. Medicine & Health. 2009;92:359.
2. Grassi D, Ferri L, Desideri G, Di Giosia P, Cheli P, Del Pinto R, Properzi G, Ferri C. Chronic hyperuricemia, uric acid deposit and cardiovascular risk. Curr. Pharma. Design. 2013;19:2432-2438.
3. Collin A. Oxidative DNA damage, antioxidants and cancer. Bio-Essays. 1999;21:238-246.
4. Nose K. Role of reactive oxygen species in the regulation of physiological functions. Biological and pharmaceutical bulletin. 2000;23:897-903.
5. Kuhn M, Letunic I, Jensen LJ, Bork P. The SIDER database of drugs and side effects, Nucl. Acids Res. 2016;44:1075-1079.
6. Mikaili P, Shayegh J, Asghari MH, Sarahroodi S, Sharifi M. Currently used traditional phytomedicines with hot nature in Iran. Annals Biol Res. 2011;2:56-68.
7. Lim TK. *Foeniculum vulgare*, in edible medicinal and non-medicinal plants. Springer Netherlands. 2013.
8. Senatore F, Oliviero F, Scandolera E, Taglialatela-Scafati O, Roscigno G, Zaccardelli M, De Falco E. Chemical composition, antimicrobial and antioxidant activities of anethole-rich oil from leaves of selected varieties of fennel [*Foeniculum vulgare* Mill. ssp. vulgare var. azoricum (Mill.) Thell]. Fitoterapia. 2013;90:214-219.
9. Badgujar SB, Patel VV, and Bandivdekar AT. *Foeniculum vulgare* Mill: A Review of Its Botany, Phytochemistry, Pharmacology, Contemporary Application, and Toxicology. Bio Med Res Inter. 2014;42674-42706.
10. Ozbek H, Ugras S, Dulger H, Bayram I, Tuncer I, Ozturk G, Ozturk A. Hepatoprotective effect of *Foeniculum vulgare* essential oil. Fitoterapia. 2003;74:317-319.
11. Akram M, Usmanghani K, Ahmed I, Azhar I, Hamid A. Comprehensive review on therapeutic strategies of gouty arthritis. Pakistan J Phar Sci. 2014;27:1575-1582.
12. Grover S, Malik C, Hora A, Kushwaha HB. Botany, cultivation, chemical constituents and genetic diversity in fennel (*Foeniculum vulgare* Mill): A review. Int J Life Sci. 2013;2:128-139.
13. Noro T, Oda Y, Miyase T, Ueno A, Fukushima S. Inhibitor of xanthine oxidase from flowers and buds of *Daphne genkwa*. Chem Phar Bulletin. 1983;31:3984-3987.
14. Teimoori-Boghsani Y, Bagherieh-Najjar MB, Mianabadi M, Mohseni M. Summer savory (*Satureja hortensis*) extract inhibits xanthine oxidase. J Med Plants & By-Products. 2015;4:25-30.
15. Benzene I, Strain J. Ferric reducing / antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous of total antioxidant power and ascorbic acid concentration. Methods Enzymol. 1999;299:15-27.
16. Mita S, Murano N, Akaike M, Nakamura K. Mutants of Arabidopsis thaliana with pleiotropic effects on the expression of the gene for beta-amylase and on the accumulation of anthocyanin that are inducible by sugars. Plant J. 1997;11:841-851.
17. Lichtenthaler H. Chlorophylls and carotenoids; pigments of photosynthetic membranes. Method Enzyme. 1987;148:350-382.
18. Kochert A. Carbohydrate determination by phenol-sulfuric acid method. In: J.A. Hellebust and J.S. Craige, Editors, Handbook of physiology and biochemical methods. Cambridge University Press, London. 1978.
19. Meda A, Lamien C, Romito M, Millogo J, Nacoulma O. Determination of the total phenolic, flavonoid and pralin contents in Burkina Fasan honey, as well as their scavenging activity. Food Chem. 2005;91:571-577.
20. Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Analysis. 2002;10:178-182.
21. Owen R, Mier A, Giacosa W, Hull B, Spiegelhalder, Bartsch H. Phenolic compounds and squalene in olive oils: the concentration and antioxidant potential of total phenols, simple phenols, secoiridoids, lignansand squalene. Food Chem. Toxic. 2000;38:647-659.
22. Amat N, Umar A, Hoxur P, Anaydulla M, Imam G, Aziz R, Upur H, Kijjoa A, Moore N. Traditional Uighur Medicine Karapxa decoction, inhibits liver xanthine oxidase and reduces serum uric acid concentrations in hyperuricemic mice and scavenges free radicals in vitro. BMC Com. & Alt. Med. 2015;15:131-139.
23. Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agri-industrial by-products:

Antioxidant activity, occurrence, and potential uses. Food Chem. 2006;99:191-203.
24. Pietta PG. Flavonoids as antioxidants. J Nat Prod. 2000;63:1035-1042.
25. De Marino S, Gala F, Borbone N, Zollo F, Vitalini S, Visioli F, Iorizzi M. Phenolic glycosides from *Foeniculum vulgare* fruit and evaluation of antioxidative activity. Phytochem. 2007;68:1805-1812.
26. Li H, Deng Z, Zhu H, Hu C, Liu R, Young JC, Tsao R. Highly pigmented vegetables: Anthocyanin compositions and their role in antioxidant activities. Food Res Int 2012;46:250-259.
27. Tummeleht L, Mägi M, Kilgas P, Mänd R, Hõrak P. Antioxidant protection and plasma carotenoids of incubating great tits (*Parus major* L.) in relation to health state and breeding conditions. Comparative Biochemistry and Physiology Part C: Toxicol & Pharmacol. 2006;144:166-172.
28. Britton G, Liaaen-Jensen S, Pfander H (Eds.). Carotenoids volume 5: nutrition and health (Vol. 5). Springer Science & Business Media. 2009.
29. Chen H, Yan X, Zhu P, Lin J. Antioxidant activity and hepatoprotective potential of agarooligosaccharides *in vitro* and *in vivo*. Nutr. J. 2006;5:31-43.
30. Faudale M, Viladomat F, Bastida J, Poli F, Codina C. Antioxidant activity and phenolic composition of wild, edible, and medicinal fennel from different Mediterranean countries. J Agric & Food Chem. 2008;56:1912-1920.
31. Cos P, Ying L, Calomme M, Hu J, Cimanga K, Van Poel B, Pieters L, Vlietinck A, Vanden Berghe D. Structure-activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. J Nat Prod. 1998;61:71-76.
32. Lin C, Hsu Y, Lin T. Antioxidant and free radical scavenging effects of the tannins of *Terminalia catappa* L. Anticancer Research. 2001;21:237-243.
33. Battelli MG, Bolognesi A, Polito L. Pathophysiology of circulating xanthine oxidoreductase: New emerging roles for a multi-tasking enzyme. Bioch. et Bioph. Acta. 2014;1842:1502–1517.