



Persian Walnut Composition and its Importance in Human Health

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Published Online December 3,
2017

Keywords: Walnut,
Antioxidant, Fatty acids,
Folk medicine



Abstract

Persian walnut (*Juglans regia* L.) is a nutritionally rich and also popular nut which is largely consumed throughout the world. In addition to dry fruits (nuts), green walnuts, kernels, shells, green walnut husks (epicarp), barks and leaves have been applied in both cosmetic and pharmaceutical industries. Since ancient times, it has been used in folk medicine for treatment of several diseases and disorders such as diabetes, cancer, skin disease and infections, rheumatoid arthritis. In recent decades, many experimental researches have been done on the composition and characteristics of different parts of Persian walnut tree. These researches showed that walnut has many nutritious components such as phenolic component, unsaturated fatty acids and minerals essential for human health. Using walnut in folk medicine and identification of beneficial components of this fruit prompted many scientists to investigate properties that have traditionally been attributed to this fruit and the researches done in this area showed that walnut has effective antioxidant, antimicrobial, antitumor and anti-inflammatory properties that make it a promising drug for associated diseases.

Received February 28, 2017; Revised October 7, 2017; Accepted October 8, 2017

Introduction

The genus *Juglans* is comprised of 21 species, among which the English or Persian walnut (*Juglans regia* L.) is the most economically important cultivated species in worldwide and has been consumed for the human nutrition purposes since ancient times and provides macronutrients and micronutrients, and also has many bioactive constituents.¹ Persian walnuts were probably domesticated in Iran and Afghanistan and then entered Eastern Europe, China and Russia by ancient tribes and were grown in some cold areas of the United States and it was demonstrated that they are genetically diverse.^{2,3} Persian walnut is an economically prominent species cultivated worldwide for providing its wood compounds and nuts. The major producers of Persian walnut are China, Iran, the United States and Turkey.⁴ This fruit is placed on FAO list of crucial plants for its nutritive value.⁵ Green walnuts, kernels, bark, shells and leaves have been employed in the pharmaceutical and cosmetic parts.⁶

Antioxidant Compositions

Antioxidant components are molecules that prevent or delay oxidation and scavenge free radicals (molecules that have unpaired electrons and initiates oxidation) that may damage cellular molecules and structures.⁷ Today, there is growing interest towards antioxidants from plants which can be used as additive instead of synthetic antioxidants

that have been proved to be carcinogenic.^{1,6,8} On the other hand, antioxidant components in diet play an important role in human health because they are able to neutralize reactive oxygen species in body which are kind of free radicals.⁹ Nuts are important dietary items that have significant amount of antioxidant components. Vinson and Cai analyzed 9 different kinds of nuts for the content of phenol compounds and among these different nuts, walnut had the highest content of free and total phenolic components as well as the highest antioxidant capacity.¹⁰ Walnut is classified as the second most consumable fruit for the high level of antioxidants.¹¹ Antioxidant components in Persian walnut kernel virtually belong to phenolic component and there are very low amounts of flavonoids and iso-flavonoids in this nut. However, other parts of this fruit that are not edible such as husk, hull, shell and leaves have a high amount of flavonoid components. Phenolic components are not confined to kernel of Persian walnuts and reports show that other parts of walnut such as leaves, pellicle, husk and microshoots have even more phenolic component compared to kernel and thus can be used as antioxidant in food industry and pharmaceutical cases.^{12,13} The most abundant phenolic components that have been identified in Persian walnut include syringic acid, ferulic acid, coumaric acid, vanillic acid, myricetin and juglone.¹⁴ Juglone (5-Hydroxy 1,4-naphthoquinone) is a specific phenolic compound that does not exist

in other nuts and is abundant in walnut, especially in green parts of walnut such as leaves, fruit hulls, stem and roots.¹⁵ Phenolic content varies in different parts of Persian walnut. It is believed that the walnut pellicle is the most valuable source of walnut phenolics. In a study, Akbari et al analyzed different parts of walnut fruit among 6 genotypes of *Juglans regia* L. in terms of phenolic component and antiradical capacity. All the genotypes had the highest amount of phenolic and flavonoid component in pellicle.¹² The highest content of phenolics found in leaves was in May and July.¹⁶ The amounts of total phenolic components of different parts of walnut were tabulated in Table 1. The percentage of phenolic component in walnut varies even in a specific genotype depending on several factor such as time period and geographic origin, climatic conditions, farming practices, cultivar choice and picking date, and some agricultural factors, so, the amount of phenols fluctuates during the seasons.^{12,15,17,18} Therefore, it is possible to increase the concentration of phenolic component by improving condition in order to produce a richer source of phenolics. Even the type of solvent that is used for extraction of phenolic component could influence the amounts that are reported.¹⁹ Toasting and heating nut have adverse effect on antioxidant capacity of nut and so it is important to be served raw.²⁰ The concentration of juglone in the fruit was significantly higher compared to other phenols in the all evaluated cultivars, in studies by Colaric et al.²¹

Lipid Component

Walnut kernels contain approximately 60% oil in general. Depending on the cultivar type, its location of growth and irrigation technique, the content of walnut oil may vary from 50% to 70%. The age of branches influences

the physicochemical composition of walnut oil, with increasing the age of the branch, oil content increases.³¹ The oil has a high amount of neutral lipids (96.9% of total lipids) and low amount of polar lipids (3.1% of total lipids). The neutral lipid fraction consists mainly of triacylglycerides whereas the polar lipids mainly consist of sphingolipids. However, free fatty acids, and monoacylglycerols, diacylglycerols, sterol esters, sterols and phosphates are contents which exist only in minor quantities. Triacylglycerides from walnut oil are rich in oleic (18:1 n-9), linoleic (18:2 n-6) and linolenic (18:3 n-3) acids. Accordingly, this fruit has a high concentration of omega-6 and omega-3 polyunsaturated fatty acids (PUFA), which are considered as essential fatty acids since the human body cannot synthesize them.³² Synthesis of the important long-chain omega-3 fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in the human body, needs α -linolenic acid as the precursor. The percentage of fatty acids in Persian walnut oil varies widely. The oleic acid content of the oils ranges from 12.7% to 34%, linoleic acid ranges from 49.7% to 72%, linolenic acid varies from 9% to 25%, the saturated palmitic is between 5.24% to 8.2% and stearic ranges from 1.4%-2% to 5%.^{33,34} It was supposed that Persian walnut is able to prevent or alleviate fatty liver alongside the Iranian *Hibiscus esculentus*.³⁵

The ratios of these fatty acids are important in terms of their economic and nutritional value. For example, oils with lower linoleic and linolenic acid contents may have a longer shelf life while oils with higher levels of polyunsaturated fatty acid are more desirable since they have potential health benefits.^{33,34}

Among vegetable oils, walnut oil has the highest amounts of PUFAs (up to 78% of the total FA content).

Table 1. The Walnut Components and Their phenolic levels

	Total Phenolic	Ref.	Phenolic Component
Kernel	15540 EAG 7300 EAG 145	12,23,24	Chlorogenic acid, ferulic acid, caffeic acid, gallic acid, synaptic acid, ellagic acid, protocatechuic acid vanillic acid, coumaric acid, elagic acid, syringic acid, myricetin juglone 5-O-cafeoylquinic ^{14,22}
Shell	2300 EAG 1804	12,16	2Methoxy phenol, 1,2 benzenediol, vanillin 3,4 di Methoxy phenol ²⁵
Hull	4700 met 37400 2468	12,16	Chlorogenic acid, syringic acid, ferulic acid, gallic acid, caffeic acid, sinapic acid, ellagic acid, protocatechuic acid, vanillic acid, catechin, epicatechin, myricetin and juglone ²⁶
Husk	3428.11	27	Chlorogenic acid, ferulic acid, caffeic acid, sinapic acid, ellagic acid, protocatechuic acid, gallic acid, syringic acid, vanillic acid, catechin, epicatechin, myricetin and juglone, coumaric acid, ferulic acid, juglone ¹⁴
Leave	2136	16	Juglone p-coumaric acid, 5-O-cafeoylquinic acid, 3-O-coumaroylquinic acid, 4-O-p-coumaroylquinic acid, 3-O-caffeoylquinic acid, quercetin 3-O- galactoside (the major compound), quercetin 3-O- arabinoside, quercetin 3-O-pentoside derivates, quercetin 3-O-xyloside, quercetin 3-O-ramnoside and kaempferol 3-O-pentoside ferulic acid, coumaric acid, vanillic acid, elagic acid, myricetin and juglone, rutin ^{15,28}
Bark	3483	30	Gallic acid, chlorogenic acid, naphtho-resorcinol, resorcinol, vanillic acid, quercetin dihydrate, p-coumaric acid, trans-cinnamic acid and catechine hydrate ²⁹
Pellicle	5205	12	Ellagic acid, gallic acid methyl gallat, tannic acid, caffeic acid, p-coumaric acid ¹²

Abbreviation: EAG: estimated average.

All values are represented as mg per 100 g walnut

The FA composition of walnut oil generally resembles that of soybean oil, but the former oil contains a greater concentration of linolenic acid. Most nuts are rich in oleic acid while walnut also contains high amounts of 2 polyunsaturated fatty acids, linoleic acid and α -linolenic acid.³⁶

Walnut oil is not suitable for cooking or heat treatment of products due to the presence of unsaturated fatty acids (70%). Their oxidation results in the appearance of unwanted taste and odor, but because of its harsh and distinctive flavor, the fruit is often used as an additive for flavoring baked goods and some sauces. It also can be used as a good flavor in salads or it can be mixed with flavored oils to produce a subtle taste.³⁷ The amount of total sterols in walnut oil ranges from 120 to 200 mg/100 g oil which contains β -sitosterol (85%), $\Delta 5$ -avenasterol (7%) and campesterol (4%). A specific amount of cholesterol, stigmasterol, $\Delta 7$ -stigmasterol and $\Delta 7$ -avenasterol has also been reported in walnut oil.³⁸

Mineral Elements

Walnut is proved to be a proper source for dietary minerals, walnut kernel is rich in potassium (K), magnesium (Mg), phosphorus (P) and iron (Fe), and it also contains sodium (Na), calcium (Ca), zinc (Zn) and copper (Cu) modestly.^{39,40} A large number of enzymes need iron (Fe) to do their job. CA is the major component of bone and assists in teeth development. K is the third most abundant mineral in the human body with electrolyte function. This mineral is needed for keeping heart, brain, kidney, muscle tissues and other important organs of human body in a good state. Therefore walnut is a good food item in term of essential minerals (Table 2).^{41,42}

Other Compounds

Walnut is a perfect source of vitamin structures such as vitamin E in 2 forms: alpha-tocopherol and gamma-tocopherol, retinol (vitamin A), folate, D-3, ergosterol, β -sitosterol, and stigmasterol.⁴⁶ It contains 18% to 24% protein based on dry weight state. Walnut protein products are thus considered as suitable resources of essential amino acids applied in human food products. Mao et al analyzed walnut proteins and classified them into 4 groups as follows: Glutelin (soluble in dilute NaOH), globulin (salt-soluble), albumin (water-soluble) and prolamin (alcohol-soluble), and based on their percentage, most of walnut proteins belong to glutelin group.⁴⁷ In another study, it was reported that some peptides resulted from hydrolyzing the walnut proteins have antioxidant activity and could be used as antioxidant components.⁴⁸ This fruit also has a lot of free amino acids. The total amino acid composition of the walnut kernel is known by a relatively high amount of alanine.⁴⁹

Walnut in Folk Medicine

Today, in spite of remarkable advances in modern

Table 2. Mineral Elements of Persian Walnut and Their Concentrations

Mineral Element	Values	Ref
K	358	43
	414	44
	372.69	42
	340	41
Mg	153	43
	216	44
	140	42
	115	41
Ca	125	43
	61	44
	120	42
	72	41
Fe	3.8	43
	4.6	44
	3.2	41
P	362	45
Na	4.4	43
	6.4	45
	7.4	44
	11.6	41
Cu	3.8	45
	2.12	43
	2.2	44
	1.2	41

All values are represented as mg per 100 g walnut.

medicine, still there is an increasing tendency towards herbal remedies among those seeking for traditional therapies. Historically all parts of Persian walnut such as stem, bark, leaves, fruits, kernels, and also kernel oil are consumed in folk medicines for the treatment of a variety of health disorders. The leaves of this species are reportedly a source of healthcare maintaining compounds and have also been traditionally used because of its antimicrobial, anthelmintic, anti-inflammatory, astringent, keratolytic, antiseptic, antidiarrheal, hypoglycemic, tonic, depurative, carminative activity.¹³ The leaves have also been used in decoctions and other preparations for the treatment of sinusitis, cold and stomach ache, pharyngitis and for reducing fever and rheumatic pain as a sedative. They are also remedies for dermal inflammation, acne, warts, excessive perspiration of the hands, feet and also for the treatment of scrofula and chronic eczema and they have also been used for washing wounds and burns. The leaves are used to treat scalp itching and dandruff as well as skin disorders. It also has a high potential of anti-atherogenic and a remarkable osteoblastic activity.^{50,51}

Walnut green husks have been employed in traditional Arabic Palestinian herbal medicine to treat several human diseases such as skin diseases caused by syphilis and fungi, wart, stomachache, anemia, and they were reported to have antihelmintic, astringent, and nerve tonic properties.⁵²

The bark, branches and husk of the green walnut have been used as a traditional medicine for the treatment of gastric, lung and liver cancer for a long time in China, Korea and the northeastern region of Mexico. In Tunisia, the bark is used as miswaks for teeth cleaning. In Nepal, the bark paste is used for the treatment of arthritis, toothache, skin

diseases and hair growth. The shell of this fruit is used in Calabria folk medicine for treating malaria.

Traditionally, the seeds of this medical plant are widely used for the treatment of venous insufficiency, hemorrhoidal symptoms, and they also have anti-diarrheal, anti-diabetic and depurative properties.

In Iran, the internal septum of walnut has been used in folk medicine for the treatment of diabetes.

The stem bark is believed to be alterative, anthelmintic, astringent bactericidal, depurative, digestive, diuretic, stimulant, tonic, laxative, detergent and insecticidal.⁷ The walnut oil is a compound for dry skin creams, and meanwhile, anti-aging and anti-wrinkle products since it has moisturizing features in addition to free radical scavenging capacity.

Pharmacological Studies

Anti-diabetic Activity

In recent years, a variety of studies have been done on the extracts of several parts of walnut and their effects on a variety of ailments were analyzed. Most of these researches have to do with hypoglycemic effect of walnut extracts on streptozotocin-induced diabetic animals and diabetic patients. Several animal studies have shown that *J. regia* leaf extract is able to ameliorate hyperglycemia related to diabetes, decrease glycosylated hemoglobin, increase insulin level and even prevent toxic effects of streptozotocin in rats.^{52,53} In addition, it is reported that *J. regia* leaf extract favorably affects pancreatic cells in the alloxan-induced diabetic rat at a concentration of single dose (150 mg/kg) dissolved in saline.⁵⁴

Results show that Internal septum extract of walnut fruit and the hydroalcoholic extract of walnut male flowers are capable of decreasing blood glucose, AST and ALP enzymes in the treated diabetic rats compared to the non-treated ones ($P < 0.001$).^{55,56} Studies on diabetic patients demonstrated that intake of walnut leaf extracts 2 times a day for 3 months decreases the concentration of serum glucose and improves both types of diabetes without any tangible side effects.^{52,57}

Studies have demonstrated that walnut extract and its polyphenolic components, especially casuarictin⁷ and tellimagrandin II were proved to be strong competitive inhibitor against amylase and sucrase. Tellimagrandin I,⁶ tellimagrandin II¹⁰ and casuarictin have an inhibitory effect on maltase and sucrose.⁵⁸

Anticancer Activity

The leaves extract of the *J. regia* showed a strong and dose-dependent anti-proliferative function against prostate cancer cells in human by inducing the apoptosis through some concomitant alterations in cell cycle phase distribution as it contains an anti-cancer agent such as ellagitannins at concentrations ranging from 100 to 200 mg/kg.⁵¹

In addition, experiments employing human renal cancer

cell lines of A-498 and 769-P and also the colon cancer cell line, Caco-2, depicted antiproliferative efficiency of walnut leaves extracts.⁵⁹

Apigenin, a flavonoid component that exists in green walnut husks, reported to be an anticancer agent without inducing apoptosis in the human peripheral blood mononuclear cell.⁵⁸

Anti-amnesia Activity

In a study by Harandi et al, it was reported that consumption of walnuts at doses of 6% and 9% could significantly restore the scopolamine-induced memory impairment in rats and prevent scopolamine neurotoxicity by decreasing activity of acetylcholine esterase in the whole brain⁶⁰ and it was shown that antioxidant peptides from walnut hydrolysate have significant potential properties in the improvement of learning and memory in D-galactose induced memory impairment in rats.⁶¹

Anti-inflammatory Activity

In a study by Nabavi et al, the anti-inflammatory activity of ethanol extracts of Persian walnut flower was demonstrated against the carrageenan-induced model of hind paw edema in mice, while not inducing the gastric damage.⁶² Hosseinzadeh et al showed that *J. regia* leaves extract has an anti-nociceptive effect via non-opioid receptors and anti-inflammatory effect on acute and also chronic inflammation. Therefore, the extracts from flowers and leaves of Persian walnut have the potential for promising analgesic and anti-inflammatory activities toward diseases such as rheumatoid arthritis.⁶³

Antioxidant Activity

Several surveys have been conducted to assess the antioxidant properties of the *J. regia* organs using different solvents and different ways such as reducing potential, superoxide radical scavenging assay, nitric oxide, 1-diphenyl-2-picrylhydrazyl (DPPH) and TEAC radical scavenging assay. These studies revealed that almost all part of walnut, especially its shell and hull have (probably for protecting the kernel) significant antioxidant activity which is related to the presence of phenolic and flavonoid components. Antioxidant activity of walnut extracts is linked to useful health function of phenolic antioxidants because of their inhibitory features against the progress of many diseases related to oxidative stress, such as inflammatory bowel syndrome, cardiovascular, degenerative diseases and Alzheimer disease.^{6,10,24,48}

Some authors indicated that walnut extract can inhibit poisoning which is induced in rats' testes.⁶⁴ The effect of polyphenols extracted from walnut on the oxidative stress induced by type 2 diabetes was investigated in the rats and it was exhibited that this material has antioxidant activities, both in vivo and in vitro. It is also showed that oral use of walnut can increase melatonin and total antioxidant capacity in the blood and protect

against certain types of cancer and may reduce the risk of cardiovascular and cerebrovascular events because of the presence of antioxidants capable of cleaving free radicals, and thus reducing oxidative damage induced in cellular macromolecules (proteins, lipids and nucleic acids).^{53,65,66}

Antibacterial Activity

Because of the rapid appearance of multiple drug-resistant bacteria, there is an urgent need for more studies on novel antimicrobial substances. Investigations showed that *Juglans* extract could be used favorably for the treatment of skin diseases, dental plaque, nosocomial pneumonia, urinary tract and community infections because of its strong antimicrobial activity. Bark and leaves extracts of *Juglans regia* found to have antibacterial activity against gram-positive and gram-negative bacteria in a dose-dependent manner. It was found that Persian walnut leaves have antibacterial effects on *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus sanguinis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Agrobacterium tumefaciens*, *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella* spp, *Propionibacterium acne*, *Staphylococcus epidermidis*, *Mycobacterium tuberculosis*, *Listeria monocytogenes*, *Listeria ivanovii*, *Listeria murrayi* and *Lactobacillus casei*.^{6,50,67,68} These findings emphasize that *Juglans regia* bark has more potent antimicrobial activity against gram-positive bacteria. However, the anti-bacterial activities of the compounds have not been developed to be used as commercial antimicrobial agents and there is a need for more investigations in this regard.

Antifungal Activity

Walnut extracts has been recognized as a protective system against pathogenic fungi: three dermatophytes, *Microsporum canis* (S14, S20, and SH41), *Trichophyton mentagrophytes* (SH13, SH1, SH8) and *T. Rubrum*, the causative agent of chalkbrood disease in bees, *Ascophaera apis*, *Candida albicans*, *Cryptococcus neoformans*, *Microsporum canis*, and *Trichophyton violaceum* (S5, SH32, SH38).²⁹

Antimicrobial activity of walnut extracts is attributed to juglone that has allelopathic effect against adjoining plants of the walnut.⁶⁹ It is reported that juglone acts as an antimicrobial agent by implementing the inhibitory effect against 3 main enzymes, cystathionine g-synthase (HpCGS), malonyl-CoA:acyl carrier protein transacylase (HpFabD), and β -hydroxyacyl-ACP dehydratase (FabZ), and it may be a promising agent against the bacterial resistance development.⁷⁰

Antiviral Activity

Walnut extract can act as an antiviral agent because the juglone has exhibited strong inhibitory effect against RNA-dependent polymerase, DNA-dependent polymerase, and DNA polymerase I activity since it can eradicate viruses

such as herpes simplex virus (HSV), Sindbis virus (SINV) and poliovirus at non-cytotoxic concentrations.⁷¹

Conclusion

The multiple aspects of usefulness conferred on the Persian walnut extracts may make it as a powerful substitute for other chemical compounds used today. However, similar to every herbal drug, more in vivo studies are needed alongside the in vitro surveys in order to confirm the results.

Authors' Contributions

SE and RJ wrote the manuscript; FN and ZZ helped in manuscript edition and data collection.

Ethical Approval

Not applicable.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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