Journal of HerbMed Pharmacology

Journal homepage: http://www.herbmedpharmacol.com

Total phenolic compounds and in vitro antioxidant potential of crude methanol extract and the correspond fractions of *Quercus brantii* L. acorn.

Ali Karimi[1], Mohammad-Taghi Moradi[2*]

[1]Faculty of Paramedicine, Shahrekord University of Medical Science, Shahrekord, Iran
[2]PhD student, Medical Plants Research Center, Shahrekord University of Medical Science, Shahrekord, Iran

ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 13 October 2014
Accepted: 24 December 2014

Keywords:
Antioxidant
Phenolic compounds
Fraction
Medicinal plants
Quercus brantii Lindl.

ABSTRACT

Introduction: Due to their roles in the maintenance of human health, phytochemicals and antioxidants in plants are raising interest. Phenolic and flavonoids are components with potential to protect against human diseases such as cardiovascular disease and cancers. *Q. brantii* is one of the traditional folk medicinal plants widely used in Iran. In this study, crude methanol extract and four correspond fractions of this plant were used to evaluate the total phenolic, total flavonoids, total flavonol, and antioxidant activities.
Methods: The identification of phenolic, flavonoid, and flavonol components and assay of antioxidant activity were carried out using standard in vitro procedures.
Results: The n-butanol fraction (376.2±7.1) had the highest amount of total phenolic compounds and the n-butanol fraction exhibited the lowest IC50 value (6.5±0.6 µg ml). There was significant relationship between the total phenolic contents and the free radical scavenging property in the four fractions in this study (R= -0.768, P< 0.01).
Conclusion: The results indicate that at least, n-butanol fraction of this plant with high phytoconstituents and less toxicity could be a promising source of medicinally important natural compound.

Implication for health policy/practice/research/medical education:
This study indicates that, at least some fractions of methanolic extract of *Q. brantii* acorn can be used as an accessible source of natural antioxidants. However, further works might be needed to the other extracts of this plant to reveal and evaluate its antioxidant potential.
Please cite this paper as: Karimi A, Moradi MT. Total phenolic compounds and in vitro antioxidant potential of crude methanol extract and the correspond fractions of *Quercus brantii* L. acorn. J HerbMed Pharmacol 2015; 4(1): 35-39.

Introduction

As an available source of innovative active biological compounds, medicinal plants are increasingly analyzed to determine the components on the structural level (1). These new compounds are pivotal for the discovery of new herbal medicines. Some kinds of side effects such as carcinogenesis have been attributed to synthetic antioxidants and thus, there is increasing interest to replace them with naturally occurring antioxidants (2,3). Antioxidant activity is one of the most important biological properties of these natural compounds. As natural antioxidants are capable of promoting food quality and stability and could act as nutraceuticals to end free radical chain reaction in biological systems, an increasing attention has been recently paid to directly evaluating the antioxidant properties of plant extracts as the source of natural antioxidants (4,5).

Many of naturally occurring antioxidants have been found to be free radical or active oxygen scavengers (6) and thus plants have been investigated for their biological activities and antioxidant properties (7-10). Flavonoides which are of the largest group of herbal polyphenols (11) contain tannins (12). Recent reports about antioxidant activity and polyphenol contents of herbals, used for fighting cancer, show that polyphenols, i.e. flavonoids, tannins, and phenolic acids, have antioxidant properties.

Belongs to the family of Fagaceae, Genus Quercus contains 500 species, some of which such as *Quercus brantii L.* are

***Corresponding author**: Mohammad-Taghi Moradi, Medical Plants Research Center, Shahrekord University of Medical Science, Rahmatieh, Shahrekord, Iran. Email: mtmoradi65@gmail.com

predominant in central and northern regions of Iran (13). The fruit of Oak tree is called acorn and is placed within a cup called Gland. Vitamins, nutrients, and Carbohydrates have been reported to comprise a large portion (48%-85%) of acorn components. Acron also contains considerable amounts of phenolic, tannin, catechin, epicatechin, and gallocatechin components (13-16).

There are some reports indicating different biological activities of some species of Genus Oak include Iranian Oak with use of the acorn and bark in treatment of some diseases such as chronic dermatological diseases, eczema, and varis (2,7,14,17-19). Additionally, some of the biologically active substances of acorn are utilized in preparing functional foods (20-22). To the best of our knowledge, to date, there has been no reports on the phytochemical analysis and antioxidant potential of different fractions of *Q. brantii* acorn. The main aim of this work was to evaluate phytochemical screening and antioxidant activity of both crude methanol extract and four correspond fractions of *Q. brantii* acorn.

Materials and Methods

Plant collection

The fruits of Oak were gathered from southwest region of Iran. Then, in Herbarium of Medical Plants Research Center of Shahrekord University of Medical Sciences (Iran), genus and species of the plant were identified and confirmed.

Extraction and fractionation of plant material

The fruits were harvested and powdered three times per maceration method. The plant material was dissolved in 70% ethyl alcohol and kept at room temperature for 96 hours. After that, the mixture was filtered and concentrated under nearly vacuum pressure and at 40°C using rotary evaporator. Having crude methanol extract of the plant, four fractions of the crude extract, with different polarity through in-solution isolation and using the difference in various secondary metabolites' polarity, were prepared. To isolate hexane fraction, the extract concentrated, suspended in 70% ethyl alcohol, and mixed with equal volume of normal hexane with sufficient shaking. The remaining plant material, from which the methanol was removed, mixed with distilled water and with chloroform in equal volume, shaked, dried over anhydrous sodium sulfate and used as the chloroform fraction. To prepare butanol fraction, equal volume of butanol was added to the remaining aqueous phase of the material, shaked and concentrated at 40°C and in vacuum condition. The remaining aqueous phase was concentrated, with the similar condition, mentioned above and used as aqueous fraction. The extracts were kept in sterile bottles, under refrigerated conditions, until further use.

Determination of the free-radical scavenging activity

The free-radical scavenging activity was measured by the 2,2 diphenyl-1-picrylhydrazyl (DPPH) method described by Moon and Terao, with some modification (23). Different amounts of each extract and methanol were added to a solution of 0.3 mg/mL methanolic solution of DPPH to make up a total volume of 3.0 mL. After standing for 15 min at room temperature, the absorbance was measured at 517 nm using UV–Vis spectrophotometer (UNICO 2100: USA). High absorbance of the reaction mixture indicated low free radical scavenging activity. Butylated hydroxytoluene (BHT) was used as positive control. Inhibition of free radical by DPPH was calculated as follows: Antiradical activity $(\%) = (A_{control} - A_{sample})/A_{control} \times 100$. The IC_{50} value, defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50% and was calculated based on linear regression of plots of the percentage antiradical activity against the concentration of the tested compounds (24). The experiment was carried out in triplicate and the results are average values.

Determination of total phenolic content

The total phenolic content of the crude extract and four correspond fractions of *Q. brantii* fruits was determined using Folin-Ciocalteu method (25). Briefly, 0.1 ml of each of the diluted samples was added to 0.5 ml of 10% (v/v) Folin-Ciocalteu reagent. After 3-8 min, 0.4 ml of 7.5% (w/v) sodium carbonate solution was added to the mixture. After being kept in total darkness for 30 min, the absorbance of the reaction mixture was measured at 765 nm using a UV–Vis spectrophotometer (UNICO 2100: USA). Amounts of total phenolic were calculated using a gallic acid calibration curve. The results were expressed as gallic acid equivalents (GAE) g/g of dry plant matter.

Determination of total flavonol content

The total flavonol content was measured following a previously reported method (26). Briefly, 0.5 ml of each diluted plant material was independently mixed with 1.5 ml of methanol, 0.1 ml of 10% (w/v) aluminum chloride, 0.1 ml of 1M potassium acetate, and 2.8 ml of distilled water. After incubation at room temperature for two and a half h absorbance of the reaction mixture was read at 440 nm using a UV–Vis spectrophotometer (UNICO 2100: USA). The results were expressed in mg of rutin equivalents of dry plant matter by comparison with the standard curve, which was made in the same condition. All measurements were done in triplicate and statistical analysis was done by statistical software using one-way analysis of variance (ANOVA) and the post-hoc Tukey's test. All the data are expressed as ± SEM.

Results

DPPH radical scavenging activity

Our results showed that the scavenging effect of the fractions increases as the concentration increases. The four fractions of *Q. brantii* fruit significantly reduced the DPPH. The concentration of each fraction which showed inhibition of higher than 50% were found to be significant ($P<0.05$; Table 1). The n-butanol fraction exhibited the lowest IC_{50} value (6.5±0.6 µg ml) as compared to the other

fractions under study. The crude extract, chloroform fraction, and aqueous fraction had IC_{50} values of 15±3, 12.7±1, and 22.4±1.7 µg/ml, respectively, and the n-hexane fraction showed highest value (144.54±5). The results are expressed relative to butylated hydroxytoluene (BHT), a reference standard having IC_{50} of 25.41±1.89 µg/ml. The IC_{50} values of the chloroform fraction, the n-butanol fraction, the crude extract, and the n-hexane fraction were found to be significant (P<0.05) while that of the aqueous soluble fraction non-significant (P>0.05) as compared with BHT (Table 2).

Total phenolic content

The total phenolic content of the *Q. brantii* extract and the four fractions were expressed as gallic acid equivalent and are presented in Table 2. The results showed total phenolic content of the fractions ranged from 24.4 to 376.2 mg gallic acid equivalent (mgGAE/g). Among the four fractions, the n-butanol (376.2±7.1) and the n-hexane (24.4±3.5) had the highest and the lowest amount of total phenolic compounds, respectively. The total phenolic contents of the crude extract, the chloroform fraction, the n-butanol fraction, and the aqueous fraction were significantly different from that of the n-hexane fraction (P<0.05; Table 2). There was good relation between the total phenolic contents and the free radical scavenging property in the four fractions in this study (R= -0.768, P<0.01).

Total flavonoid and flavonol content

The total flavonoid and flavonol contents of the crude extract and the four fractions of *Q. brantii* fruit were expressed as gallic acid equivalent, as rutin equivalent (mg/g) and are presented in Table 2. The most amount of flavonoid and flavonol (91.6 and 105 mg/mL, respectively) was extracted in chloroform fraction and the lease amount (5.8 and 1.1 mg/mL, respectively) was extracted in aqueous fraction (P<0.05).

Discussion

In this study, crude methanol extract and the four correspond fractions of fruit of *Q. brantii* with different polarities were prepared through in-solution isolation and using the difference in different secondary metabolites' polarity. Also, the ability of these plant materials to inhibit

Table 1. DPPH radical-scavenging activity of the crude extract and various fractions of *Quercus brantii* L. acorn.

Sample	Concentration (µg/ml)	Scavenging of DPPH radical activity inhibition (%) (main ± SEM)
Crude extract	25	80.9±0.5
	20	66.4±1.1
	15	49.7±0.9
	10	34.5±0.3
	5	19±1
n-hexane fraction	150	55.3±1.5
	125	47.5±2
	100	40±2/1
	75	37.7±.08
	50	17.4±.3
Chloroform fraction	25	91.5±1.5
	20	80.1±1.4
	15	62.5±0.8
	10	40.2±1.3
	5	22.9±2.4
n-Butanol fraction	10	75±1.5
	7.5	58.2±1.2
	5	47.3±0.3
	2.5	25.8±1.3
	1	8.6±1.2
Remaining aqueous fraction	40	86.8±2.5
	30	66.5±1.9
	20	44.3±0.7
	10	19±0.9
	5	11.8±1.4
BHT	50	90.8±1.5
	40	78.3±1.2
	30	55.5±0.7
	20	40.09±1.7
	10	22±1.06

All results are presented as mean ± standard mean error of three assays. DPPH: 1,1-Diphenyl-2-picrylhydrazyl, BHT; Butylated hydroxytoluene

Table 2. DPPH-radical scavenging activity IC_{50}, total phenolics, flavonoid, and flavonol values of the different fractions of *Quercus brantii* L. acorn.

Sample	DPPH-radical scavenging activity IC_{50}/(µg/ml)	Total phenolics (mg GAE/g)	Flavonoid content (mg/g)	Flavonol content (mg/g)
Crude extract	15±3$^{\#a}$	201.6±4	14.7±2.2^{a}	33.6±2
n-hexane fraction	144.5±5$^{\#}$	24.4±3.5	16.8±2^{a}	2.4±0.5^{a}
Chloroform fraction	12.7±1$^{\#ab}$	222.5±2.5	91.6±1.5	105±4.5
n-Butanol fraction	6.5±0.5$^{\#b}$	376.2±7.1	22±2	70.9±55
Remaining aqueous fraction	22.4±1.7	120±3.5	5.8±0.5	1.1±0.4^{a}
P-value*	<0.01	<0.01	<0.01	<0.01

All results are presented as mean ± standard mean error of three assays.
*In terms of analysis of variance; ^{a,b} values with the same letter in each column are not significantly different according to Tukey test (P< 0.05); # Significant when compared with the reference standard (BHT, IC_{50} of 25.41±1.89 µg/ml).
Abbreviations: DPPH, 1,1-Diphenyl-2-picrylhydrazyl; IC_{50}, the concentration of a sample at which 50% inhibition of free radical activity is observed; GAE, gallic acid equivalent.

free radicals was assessed using DPPH method.
Among the 4 fractions, the n-butanol (376.2±7.1) and the n-hexane (24.4±3.5) had the highest and the lowest amount of total phenolic compounds, respectively. Based on the scavenging capacity of the free radicals, the n-butanol exhibited the highest antioxidant activity with the highest percent inhibition of the DPPH radical, followed by the chloroform fraction with higher percent inhibition of the DPPH radical compared to that of BHT. Also, the n-hexane fraction contained lower percent inhibition of the DPPH radical compared to BHT and the other fractions, indicating proper relation between the total phenolic contents and the free radical scavenging property, given that as IC_{50} value decreases, the ability to inhibit free radicals increases.
Similar to our study, some authors (27,28) have demonstrated a linear relation between the content of total phenolic compounds and their antioxidant capacity of some plant extracts. Therefore, the antioxidant potential of our plant extracts most probably is due to presence of phenolic components (376.2±7.1). Additionally, as tannins have been reported to be considerable components of phenolics in *Q. brantii* fruit (12), notable antioxidant potential of our fractions could be also due to the tannins. Our results, based on the DPPH method, showed that with increasing the polarity of the extracts, their radical scavenging activity will increase. Similar findings were reported on the rate of antioxidant property of the bark of *Quercus robur* (14). In this study, the crude methanol extract of *Q. brantii* fruit and some of the fractions showed promising antioxidant activity, by inhibiting DPPH, which is related to its total phenolic content. The presence of phenols and flavonoids in the plant, as its major constituents, plays a major role in controlling antioxidants. Phenolic compounds are very important plant constituents because their phenolic groups are responsible for high antioxidant activity and their hydroxyl groups confer scavenging ability.

Conclusion

The results of this study showed that at least some fractions of methanolic extract of *Q. brantii* acorn could be used as easily accessible source of natural antioxidants. However, as some of the fractions show lower activity in DPPH and total phenol content as compared with standard, further works may be taken up to the other extracts of this plant to reveal and evaluate its antioxidant potential.

Acknowledgments

Authors are thankful to the Director of Medical Plants Research Center and to the Deputy of Research of Shahrekord University of Medical Sciences, Shahrekord, Iran for financial support.

Authors' contributions

All authors contributed equally.

Conflict of interests

The authors declare that there are no conflicts of interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

Funding/Support

Deputy of Research and Technology of Shahrekord University of Medical Sciences, Shahrekord, Iran.

References

1. Clark AM. Natural products as a resource for new drugs. Pharm Res 1996; 13(8): 1133-44.
2. Karimi A, Moradi MT, Saeedi M, Asgari S, Rafieian-Kopaei M. Antiviral activity of Quercus persica L.: High efficacy and low toxicity. Adv Biomed Res 2013; 2: 36.
3. Znati M, Hichem BJ, Cazaux S, Bouajila J. Chemical composition, biological and cytotoxic activities of plant extracts and compounds isolated from Ferula lutea. Molecules 2014; 19(3): 2733-47.
4. Ito N, Fukushima S, Hagiwara A, Shibata M, Ogiso T. Carcinogenicity of butylated hydroxyanisole in F344 rats. J Natl Cancer Inst 1983; 70(2): 343-52.
5. McClements DJ, Decker EA. Lipid Oxidation in Oil-in-Water Emulsions: Impact of Molecular Environment on Chemical Reactions in Heterogeneous Food Systems. J Food Sci 2000; 65(8): 1270-82.
6. Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. J Agric Food Chem 2001; 49(11): 5165-70.
7. Basri D, Fan S. The potential of aqueous and acetone extracts of galls of Quercus infectoria as antibacterial agents. Indian J Pharmacol 2005; 37(1): 26-9.
8. Rafieian-Kopaei M, Baradaran A, Rafieian M. Oxidative stress and the paradoxical effects of antioxidants. J Res Med Sci 2013; 18(7): 629.
9. Rafieian-Kopaei M, Baradaran A, Rafieian M. Plants antioxidants: From laboratory to clinic. J Nephropathol 2013; 2(2): 152-3.
10. Riaz T, Abbasi A, Aziz-Ur-Rehman A, Shahzadi T, Ajaib M, Khan M. Phytochemical screening, free radical scavenging, antioxidant activity and phenolic content of Dodonaea viscosa. J Serb Chem Soc 2012; 77(4): 423-35.
11. Kovacevic N. Basic Pharmakognosy. Belgrade: University of Belgrade–Faculty of Pharmacy; 2000.
12. Rakic S, Petrovic S, Kukic J, Jadranin M, Tešević V, Povrenović D, *et al.* Influence of thermal treatment on phenolic compounds and antioxidant properties of oak acorns from Serbia. Food Chem 2007; 104(2): 830-4.
13. Saffarzadeh A, Vincze L, Csapo J. Determination of the chemical composition of acorn (Quercus branti), Pistacia atlantica and Pistacia Khinjk seeds as non-conventional feedstuffs. Acta Agr Kapos. 1999; 3(3): 59-69.
14. Andrensek S, Simonovska B, Vovk I, Fyhrquist P,

Vuorela H, Vuorela P. Antimicrobial and antioxidative enrichment of oak (Quercus robur) bark by rotation planar extraction using ExtraChrom. Int J Food Microbiol 2004; 92(2): 181-7.
15. Cadahia E, Munoz L, Fernandez de Simon B, Garcia-Vallejo MC. Changes in low molecular weight phenolic compounds in Spanish, French, and American oak woods during natural seasoning and toasting. J Agric Food Chem 2001; 49(4): 1790-8.
16. Popovic BM, Stajner D, Zdero R, Orlovic S, Galic Z. Antioxidant characterization of oak extracts combining spectrophotometric assays and chemometrics. Scientific World Journal. 2013; 2013: 134656.
17. Aslani A, Emami S, Ghannadi A, Ajdari M. Formulation and physicochemical evaluation of an herbal antihemorrohid ointment from Quercus, Black cumin and Fenugreek for the treatment of internal anal hemorrhoids. J Pharm Sci Tabriz Univ Med Sci 2009; 14: 247-57.
18. Kaur G, Hamid H, Ali A, Alam MS, Athar M. Antiinflammatory evaluation of alcoholic extract of galls of Quercus infectoria. J Ethnopharmacol 2004; 90(2-3): 285-92.
19. Khosravi A, Behzadi A. Evaluation of the antibacterial activity of the seed hull of Quercus brantii on some gram negative bacteria. Pak J Med Sci 2006; 22(4): 429-32.
20. Cantos E, Espin JC, Lopez-Bote C, de la Hoz L, Ordonez JA, Tomas-Barberan FA. Phenolic compounds and fatty acids from acorns (Quercus spp.), the main dietary constituent of free-ranged Iberian pigs. J Agric Food Chem 2003; 51(21): 6248-55.
21. Rakić S. Effect of oak acorn extracts on lipide oxidation kinetics. J Agric Sci 2000; 45(2): 139-43.
22. Rakić S, Maletić RO, Perunović MN, Svrzić G. Influence of thermal treatment on tannin content and antioxidation effect of oak acorn Quercus cerris extract. J Agric Sci 2004; 49(1): 97-107.
23. Moon JH, Terao J. Antioxidant Activity of Caffeic Acid and Dihydrocaffeic Acid in Lard and Human Low-Density Lipoprotein. J Agric Food Chem 1998; 46(12): 5062-5.
24. Nahak G, Sahu RK. In vitro antioxidative acitivity of Azadirachta indica and Melia azedarach Leaves by DPPH scavenging assay. Nat Sci 2010; 8(4): 22-8.
25. Folin O, Ciocalteu V. On tyrosine and tryptophane determinations in proteins. J Biol Chem 1927; 73(2): 627-50.
26. Rabiei Z, Rafieian-kopaei M, Heidarian E, Saghaei E, Mokhtari S. Effects of zizyphus jujube extract on memory and learning impairment induced by bilateral electric lesions of the nucleus basalis of meynert in rat. Neurochemical research 2014; 39(2): 353-60.
27. Rahnama S, Rabiei Z, Alibabaei Z, Mokhtari S, Rafieian-kopaei M, Deris F. Anti-amnesic activity of Citrus aurantium flowers extract against scopolamine-induced memory impairments in rats. Neurological Sciences Neurol Sci 2014 Nov 4.
28. Shirzad H, Taji F, Rafieian-Kopaei M. Correlation between antioxidant activity of garlic extracts and WEHI-164 fibrosarcoma tumor growth in BALB/c mice. J Med Food 2011; 14(9): 969-74.