FABAD J. Pharm. Sci., 37, 17-22, 2012

RESEARCH ARTICLE

Antioxidant Activity and Total Phenolic Content of *Quercus coccifera* L.

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Antioxidant Activity and Total Phenolic Content of Quercus coccifera L.

Summary

The water and MeOH extracts of the stems of Quercus coccifera L. are screened for their radical scavenging activity against 2,2-diphenyl-1-picryl hydrazyl (DPPH), superoxide (SO) and nitric oxide (NO) radicals spectroscopically. Dose dependent radical scavenging activity was observed and the results were found to be comparable to that of known antioxidant compounds BHA (3-t-butyl-4-hydroxyanisole), ascorbic acid and quercetin. In addition, gallic acid equivalent total phenolic contents of the plants were also determined using Folin-Ciocalteau reagent.

Key Words: *Quercus, Fagaceae, Radical scavenging effect, DPPH, Nitric oxide, Superoxide, Total phenolic content*

Received: 13.11.2013
Revised: 10.12.2013
Accepted: 28.01.2014

Quercus coccifera L.'nin Toplam Fenolik İçeriği ve Antioksidan Aktivitesi

Özet

Quercus coccifera L. gövdelerinin su ve metanol ekstrelerinin radikal süpürücü aktiviteleri 2,2-difenil-1-pikril hidrazil (DPPH), süperoksit (SO) ve nitrik oksit (NO) radikallerine karşı spektroskopik olarak taranmıştır. Doza bağımlı radikal süpürücü aktivite gözlenmiş ve sonuçların BHA (3-t-bütil-4-hidroksianisol), askorbik asit ve kersetin gibi bilinen antioksidan bileşiklerle karşılaştırılabilir olduğu bulunmuştur. Ayrıca bitkilerin gallik asite eşdeğer total fenol içerikleri de Folin-Ciocalteau reaktifi ile tanımlanmıştır.

Anahtar Kelimeler: *Quercus, Fagaceae, Radikal süpürücü etki, DPPH, Nitrik oksit, Süperoksit, Total fenolik içerik*

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INTRODUCTION

The genus *Quercus* (Fagaceae) is represented by 23 taxa in the flora of Turkey, 4 of them are endemic (1). *Quercus* species have been used as antiseptic, antidiarrheal, hemostatic, wound healing, stomachic agent and against for poisoning, from such as alkaloids, copper, lead and heavy metal salts (2-4). In scientific studies, antimicrobial, antiinflammatory, gastroprotective, antioxidant, cytotoxic and antitumoral properties were found in some species *of Quercus* (4-6). *Q. coccifera* L. is native to the Mediterranean region of Anatolia and called as "kermes oak and pinar". It is used for the treatment of diabetes and diarrhea (2, 6-9). Furthermore, the decoction of this plants used for burns and wound healing (3, 10).

Previous chemical studies on the genus *Quercus* led to the identification of flavonoids, tannins and triterpenes (4). We have also reported the isolation and structure elucidation of phytochemicals such as ionon, phenols, lignans and catechin derivative from the methanolic extract of the stems with barks of *Q. coccifera* (11).

The imbalance between oxidants and antioxidants in the body, leads to oxidative stress that is being suggested as the root cause of aging and various human diseases like atherosclerosis, stroke, diabetes, cancer and neurodegenerative diseases such as Alzheimer's disease and Parkinsonism (12). Many diseases can be attributed to the oxidation of molecules such as DNA, carbohydrates, proteins or lipids, which are necessary for proper life function (13). Natural and synthetic antioxidants has been advised for use in the treatment of various human diseases. Some synthetic antioxidant compounds like butylated hydroxytoluene, butylated hydroxyanisole commonly used in processed foods. However, synthetic antioxidants have shown potential health risks such as child hyperactivity, damage to the lungs, liver, and kidneys, and toxicity, most notably possible carcinogenicity (14-16). Therefore, to find new sources of safe and inexpensive antioxidants of natural origin in order to use them in foods and pharmaceutical preparations to replace synthetic antioxidants is mandatory (17-18). In recent years, the importance of studies on antioxidants originated plants has increased.

The aim of the present study is to characterize the antioxidant capacity of *Q. coccifera* stems extracts by determining their gallic acid equivalent total phenolic content and their radical scavenging activity using different radicals: DPPH, NO and SO.

MATERIALS AND METHODS

Plant Material

Quercus coccifera L. (Fagaceae) was collected from Sertavul-Akçeşme between Mut and Konya (Middle-South Anatolia, Turkey), near roadway, 1600 m in August 2008. It was identified by Prof. Zeki Aytaç (Department of Biology, Faculty of Sciences, Gazi University). A voucher specimen has been deposited in the Herbarium of the Faculty of Pharmacy, Hacettepe University, Ankara, Turkey (HUEF 10003).

Extraction

Aqueous extract: The air-dried, powdered stems of *Q. coccifera* (50 g) were extracted with distilled water (2 x 500 mL) at 100 ˚C during 3 hours. The combined aqueous extracts were dried in vacuo and lyophilized to give water extract (4 g, 8%).

Methanolic extract: The air-dried, powdered stems *Q. coccifera* (297 g) were extracted with MeOH (4 x 2 L) at 45 ˚C during 3 hours. The combined MeOH extracts were evaporated under *vacuum* and lyophilized to give MeOH extract (21.5 g, 7.2%).

General

DPPH, nitro blue tetrazolium (NBT), sodium nitroprusside, Folin–Ciocalteu reagent, gallic acid, ascorbic acid were obtained from Sigma-Aldrich Chem Co (St. Louis, MO). 3-*t*-butyl-4-hydroxyanisole (BHA) was purchased from Nacalai Tesque Co. (Kyoto,Japan). Sulfanilamide and napthylethylenediamine dihydrochloride were obtained from Merck Co. (Darmstadt, Germany).

DPPH radical scavenging effect

The DPPH radical scavenging effect was assessed by the discoloration of methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) spectroscopically; butylhydroxyanisole (BHA) and ascorbic acid (AA) were used as standard compounds (19-20). DPPH (50 µL, 1 mM) solution was added to MeOH solution

(200 µL) of the extract or standard compounds at various concentrations. The reaction mixture was shaken vigorously and the absorbance of remaining DPPH was measured at 520 nm after 30 min. The radical scavenging activity was determined by comparing the absorbance with that of blank (100%) containing only DPPH and solvent. All the analyzes were done in triplicate. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula:

% Radical scavenging activity = [(Control absorbance–Sample absorbance) / Control absorbance] x 100

SO radical scavenging activity by alkaline DMSO method

The method of Elizabeth and Rao was used for the detection of superoxide radical scavenging activity of samples with slight modification. Briefly, a superoxide radical was generated in a non-enzymatic system. The reaction mixture containing 10 µL of NBT (1 mg/mL solution in DMSO) and 30 µL of the samples were dissolved in DMSO. 100 µl of alkaline DMSO (1 mL DMSO containing, 5 mM NaOH in 0.1 mL water) was added to give a final volume 140 µL and the absorbance was measured at 560 nm using microplate reader (21, 22).

NO scavenging activity

In order to determine NO radical scavenging activity of the extracts and references, 60 µL of serial diluted sample was added into a 96-well flat-bottomed plate. Following this, 60 µL of 10 mM sodium nitroprusside, dissolved in phosphate buffered saline (PBS), were added to each well and the plate was incubated under light at room temperature for 150 min. Finally, an equal volume of Griess reagent (1% sulfanilamide, 0.1% napthylethylenediamine dihydrochloride, 2.5% H_3PO_4) was added into each well in order to measure the nitrite content. After chromofore was formed at room temperature in 10 min, absorbance at 577 nm was measured in a microplate reader (23, 24).

Estimation of total phenolic content

Antioxidant compounds generally contain phenolic group (s) and the amount of phenolic compounds in the extract was estimated by Folin–Ciocalteu reagent. Briefly, 10 µL sample or standard (50–500 mg/L gallic acid) plus 150 µL diluted Folin–Ciocalteu reagent (1:4 reagent:water) was placed in each well of a 96-well plate, and incubated at room temperature for 3 min. Following the addition of 50 µL sodium carbonate (2:3 saturated sodium carbonate: water) and a further incubation of 2 h at room temperature, absorbance was read at 725 nm. Quantification was done based on the standard curve of gallic acid. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram extract. All tests were conducted in triplicate (25).

RESULTS AND DISCUSSION

Radical scavenging activity results against 2,2-diphenyl-1-picryl hydrazyl (DPPH), superoxide (SO) and nitric oxide (NO) radicals of the H_2O and MeOH extracts of the stems of *Q. coccifera* and controls were shown on Table 1 in addition to their gallic acid equivalent total phenolic contents.

Table 1. Radical scavenging capacity and Total phenolic content of plant extracts with references

	DPPH IC_{50} (µg/mL)	NO IC_{50} (µg/mL)	SO IC_{50} (µg/mL)	Total Phenolic Content (mg/g dry extract)
Quercus MeOH extract	43.25	128.8	12.15	136.42
Quercus H_2O extract	67.84	313.75	394.7	89.16
BHA	16.20	500.2	824.8	–
AA	13.78	513.6	68.08	–
Quercetin	12.81	92.6	11.60	–

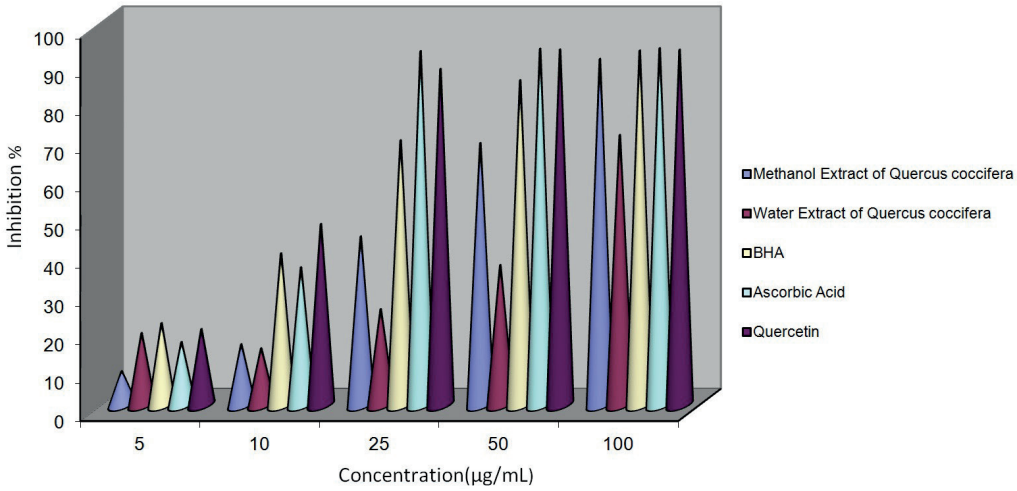

Figure 1. DPPH radical scavenging activity of *Q. coccifera* extracts

Both extracts showed dose dependent DPPH radical scavenging ability (Fig. 1). While they show good radical scavenging activity against DPPH radical, their activities are not stronger than those of standard compounds BHA, ascorbic acid and quercetin. Their IC_{50} values were found as: 67.84 µg/mL for aqueous extract and 43.25 µg/mL for MeOH extract. This activity for methanol extract of *Q. coccifera* was found very close to that of reference compounds BHA, ascorbic acid and quercetin at 100 µg/mL concentration (Fig. 1).

SO scavenging activity of the extracts were tested in the concentration range of 10–400 µg/mL and while methanol extract was found more active than the aqueous extract, ascorbic acid was the most active compound in all tested concentrations. Both extracts showed dose dependent SO scavenging activity (Fig. 2).

Our data showed significant dose dependent NO scavenging activity for both extracts then the reference compounds BHA and ascorbic acid in all tested concentration (Fig.3). Especially, methanol extract showed stronger radical scavenging activity than quercetin up to 100 µg/mL concentration and its activity was comparable to that of quercetin at higher concentrations.

And the levels of gallic acid equivalent total phenolic contents of MeOH extract of *Q. coccifera* were found very good and higher than that of H_2O extract (Table 1).

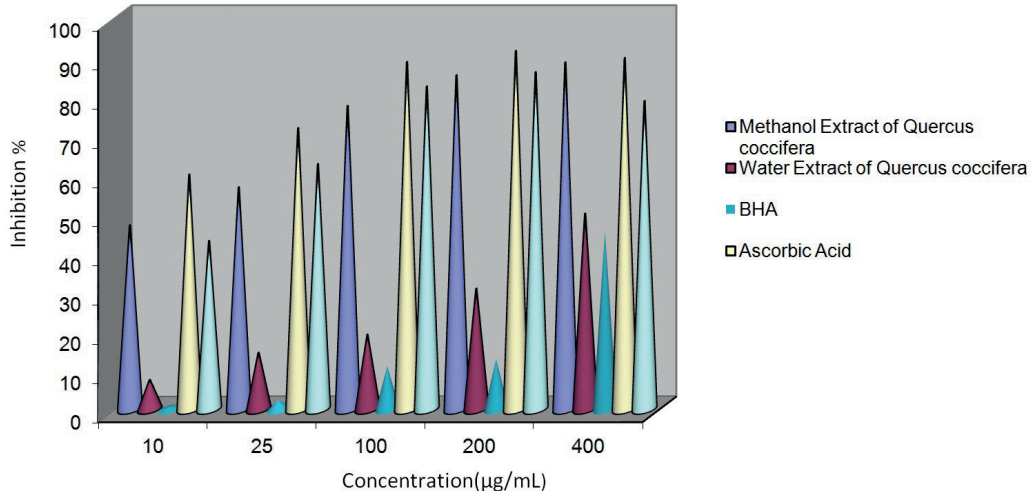

Figure 2. SO radical scavenging activity of *Q. coccifera* extracts

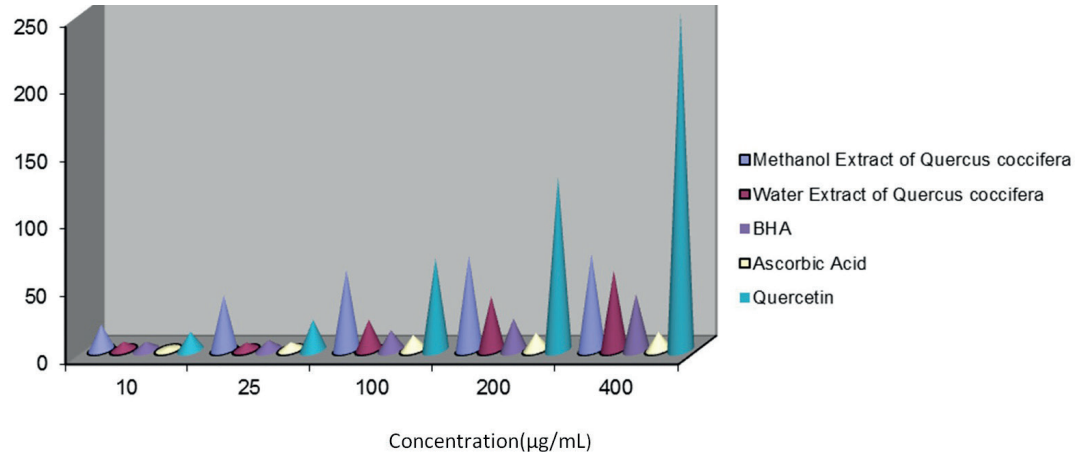

Figure 3. NO radical scavenging activity of *Q. coccifera* extracts

Different types of oxygen species such as superoxide anion, singlet oxygen and hydroxyl radicals along with peroxides and transition metals have degenerative effects to living cells and DNA in human body. The role of free radicals and reactive oxygen species are becoming increasingly important in the pathogenesis of diabetes, arteriosclerosis, cardiovascular diseases, cancer and several neurodegenerative disorders. Resent investigations have indicated that effective antioxidants are getting increasingly important in disease prevention and therapy (13, 26-27). So that the research for active antioxidants from natural sources is necessary and very important for the new drug development. In summary, these results of the present study showed that the especially methanol extract of *Q. coccifera* is a very important source for natural antioxidant agent(s). Our phytochemical and biological researches on this material are still continuing and further research is needed to clarify the active compound(s).

ACKNOWLEDGEMENT

The authors would like to thank Pharmacist İsa Arı (Mut/Mersin) for sample collection.

REFERENCES

1. Hedge IC, Yaltırık F. *Quercus* L. In: Flora of Turkey and East Aegean Islands, Ed: P.H. Davis, Vol. 7, University Press, Edinburgh, 1982.
2. Baytop T. Türkiye'de bitkilerle tedavi (Geçmişte ve Bugün), pp. 294-296, Nobel Tıp Kitabevleri Ltd. Şti., İstanbul, 1999.
3. Bremness L. Herbs, Dorling Kindersley, London, 1995.
4. Şöhretoğlu D, Sakar MK. *Quercus* türlerinin polifenolik bileşikleri ve biyolojik aktiviteleri. *Ankara Univ J Fac Pharm* 33: 183-215, 2004.
5. Şöhretoğlu D, Sabuncuoğlu S, Harput ÜŞ. Evaluation of antioxidative, protective effect against H_2O_2 induced cytotoxicity, and cytotoxic activities of three different *Quercus* species. *Food Chem Toxicol* 50: 141-146, 2012.
6. Khennouf S, Benabdallah H, Gharzouli K, Amira S, Ito H, Kim TH, Yoshida T, Gharzouli A. Effect of tannins from *Quercus suber* and *Quercus coccifera* leaves on ethanol-induced gastric lesions in mice. *J Agric Food Chem* 51: 1469-1473, 2003.
7. Goun EA, Petrichenko VM, Solodnikov SU, Kline MA, Cunningham G, Nguyen C, Miles H. Anticancer and antithrombin activity of Russian plants. *J Ethnopharmacol* 81: 337-342, 2002.
8. Ito H, Yamaguchi K, Kim TH, Khennouf S, Gharzouli K, Yoshida T. Dimer and Trimeric Hydrolyzable tannins from *Quercus coccifera* and *Quercus suber*. *J Nat Prod* 65: 339-345, 2002.
9. Alkofahi A, Atta AH, Pharmacological screening of the anti-ulcerogenic effeects of some Jordanian medicinal plants in rats. *J Ethnopharm* 67: 341-345, 1999.
10. Tuzlacı E. Şifa Niyetine, Türkiye'nin Bitkisel Halk İlaçları, Alfa Yayınları, İstanbul, 2006.
11. Şöhretoğlu D, Kuruüzüm-Uz A, Simon A, Patócs T, Dékány M. New secondary metabolites from *Quercus coccifera* L. *Rec Nat Prod* (in press).

12. Tiwari AK. Antioxidants: New-generation therapeutic base for treatment of polygenic disorders. *Current Science* 86: 1093-1102, 2004.
13. Halliwell B. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? *Lancet* 344: 721-724, 1994.
14. Tran AV. Do BHA and BHT induce morphological changes and DNA double-strand breaks in Schizosaccharomyces pombe? Scripps Senior Theses. Paper, 2013. http://scholarship.claremont.edu/scripps_theses/152
15. Song FL, Gan RY, Zhang Y, Xiao Q, Kuang L, Li HB. Total phenolic contents and antioxidant capacities of selected Chinese medicinal plants. *Int J Mol Sci* 11: 2362-2372, 2010.
16. Cuzzocrea S, Riley DP, Caputi AP, Salvemini D. Antioxidant Therapy: A new pharmacological approach in shock, inflammation, and ischemia/reperfusion injury. *Pharmacol Rev* 53: 135-159, 2001.
17. Lee J, Koo N, Min DB. Reactive oxygen species, aging, and antioxidative nutraceuticals, comprehensive. *Rev Food Sci Food Safety* 3: 21-33, 2004.
18. Kah R, Kappus H. Toxicology of the synthetic antioxidants BHA and BHT in comparison with the natural antioxidant vitamin E. *Z Lebensm-Unters.-Forsch* 196: 329–338, 1993.
19. Hatano T, Edamatsu R, Hiramatsu M, Mori A, Fujita Y, Yasuhara T, Yoshida T, Okuda T. Effects of the interaction of tannins with co-existing substances. VI. Effects of tannins and related polyphenols on superoxide anion radical and on 1,1-diphenyl-2-picrylhydrazyl radical. *Chem Pharm Bull* 37: 2016–2021, 1989.
20. Jensen SR, Gotfredsen CH, Harput US, Saracoglu I. Chlorinated iridoid glucosides from *Veronica longifolia* and their antioxidant activity. *J Nat Prod* 73: 1593–1596, 2010.
21. Elizabeth K, Rao MNA. Oxygen radical scavenging activity of curcumin. *Int J Pharm* 58: 237–240, 1990.
22. Srinisavan R, Chandrasekar MJN, Nanjan MJ, Suresh B. Antioxidant activity of *Caesalpinia digyna* root. *J Ethnopharmacol* 113: 284–291, 2007.
23. Hensley K, Mou S, Pye QN. Nitrite determinationby colorimetric and fluorometric griess diazotization assays methods in pharmacology and toxicology, In: Methods in biological oxidative stres, Eds: K. Hensley, R. A. Floyd, pp. 185-193, Humana Press Inc., Totowa, NJ, 2003.
24. Tsai P, Tsai TH, Yu C, Ho SC. Comparison of NO-scavenging and NO-suppressing activities of different herbal teas with those of Green Tea. *Food Chem* 103: 181–187, 2007.
25. Dominguez M, Nieto A, Marin J C, Keck AS, Jeffery E, Cespedes CL. Antioxidants activities of extracts from *Barkleyanthus salicifolius* (Asteraceae) and *Penstemon gentianoides* (Scrophulariaceae). *J Agric Food Chem* 53: 5889–5895, 2005.
26. Asongalem EA, Foyet HS, Ekobo S. Antiinflammatory, lack of central analgesia and antipyretic properties of *Acanthus montanus* (Ness) T. Anderson. *J Ethnopharmacol* 95: 63–68, 2004.
27. Willis MS, Wians FH. The role of nutrition in preventing prostate cancer: A review of the proposed mechanism of action of various dietary substances. *Clin Chim Acta* 330: 57–83, 2003.