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Study on antibacterial and antioxidant activity of Oak gall (*Quercus infectoria*) extracts from Iran

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Abstract

The antioxidant and antibacterial are group from food additive that use on food as preservative. The objective of this study was to determine antioxidant and antibacterial activity of *Quercus infectoria* galls the using different *in vitro* methodologies. The extracts of aquatic, ethanolic and methanolic, at a concentration from 300, 600 and 1200 µg/ml, showed a significant antibacterial effect expressed as minimum inhibitory concentration (MIC) against Gram-positive bacteria. In particular, staphylococcus arouse (MIC=300 µg/ml) and *Bacillus cereus* (MIC=600 µg/ml) were the most inhibited. The antioxidant activity were determined by the 2,2-diphenylpicrylhydrazyl (DPPH)assay and a β-carotene bleaching assay, and compared with that of butylatedhydroxyl toluene (BHT).The data were expressed as the mean ± the standard deviation and they were statistically analyzed by SPSS software using ANOVA ($P<0.05$). The results showed that among all the solvent extracts, water extract of *Quercus infectoria* galls had high antioxidant activities as measured by DPPH scavenging (30/15±0.83 µg /ml) and β-carotene linolic acid (89/4±1.11/ml). These parameters for BHT were 5±0.25 and 7.4±0.3 µg/ml respectively.

Keywords: extracts, *Quercus infectoria* galls, antioxidant activity, antibacterial activity

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Introduction

Food-borne pathogenic are group of micro-organisms that cause food-borne disease thus, the research for finding effective drugs against this infection is necessary. Prevalence of food-borne disease caused by Food-borne pathogenic has increased worldwide and has become a major cause of mortality in individuals with impaired immune systems in developing countries (Shariatifar et al., 2014). Therefore, urgent need to monitoring antimicrobial resistance by improved antibiotic use and reduce hospital cross-infection, but the development of new antibiotics (natural) must be continued to maintain the effectiveness of antimicrobial therapy of primary importance (French, 2005). In developing countries, the World Health Organization estimates that about three-quarters of the population relies on plant-based preparations used in traditional medicine for primary health care as a fundamental human needs. Therefore, some herbs have been evaluated for antimicrobial activity may be used to treat a variety of diseases and microbial origin (Paniagua-Zambrana).

Oxidative stress is an important factor in the patho-physiology of pathological conditions, including cardiovascular dysfunction, atherosclerosis, inflammation, cancer, drug toxicity, reperfusion injury and neurological disease (Bandyopadhyay et al., 1999). Different parts of

plants, including fruits, leaves; have a wide range of phenolic compounds, vitamins, terpenoids and some other endogenous metabolites, which are rich in antioxidant activity. By controlling free radicals, antioxidants can reduce fibrosis process. Antioxidants as protective agents may reduce oxidative damage in humans are considered. Antioxidants occur naturally in many fruits and are able to neutralize free radicals by donating an electron and convert them into harmless molecules (Augustyniak et al., 2010). Antioxidants that can quench reactive free radicals can prevent the oxidation of other molecules and may, therefore, have health effects in the prevention of degenerative diseases. In addition, it has been reported that an inverse relationship between dietary intake of foods rich in antioxidants and incidence human disease (Kim et al., 2003).

Quercus infectoria is a small tree native of Greece, Asia Minor and Iran. *Quercus* is a plant genus in the family of Fagaceae. This species is generally known under the name baloot in Iran and are commonly used as medicinal plant. In traditional medicine from *Quercus infectoria* is used in the treatment of intertrigo, impetigo, eczema, haemorrhages, chronic diarrhea and dysentery (Ren and Chen, 2007). *Quercus infectoria* is a small tree or shrub of about to 4 to 6 feet tall, which grows on mountain such as Kurdistan and West Azerbaijan. *Quercus infectoria* is a plant which grows wild in abandoned areas such as Iran, Iraq, Turkey and Syria (Hasmah et al., 2010). Using of *Quercus infectoria* extracts in food preparations, nutraceuticals or cosmetic anti-aging formulations may be promising. Hence, the objective of this study was to assess the antimicrobial activities and antioxidant activity of *Quercus infectoria* galls in *in vitro*. This study was designed to examine antibacterial and antioxidant activities of the extracts obtained from *Quercus infectoria* galls that have been traditionally used as general health supplements.

Materials and Methods

Plant materials

The galls of *Quercus infectoria* were collected from the Kurdistan, Iran, during Sep'2013. The taxonomic identification of plant materials was confirmed by herbarium of medicinal plants, Tehran University of Medical Sciences, Iran.

Preparation extracts

First, ethanol and methanolic extract was prepared according to the method proposed by Shyamala et al. For this purposes, 10 g of powdered leaves of *Quercus infectoria*were extracted in soxhlet apparatus with 100 ml of alcohol (HPLC grade) until the extraction solvent became colorless. The extraction was repeated twice at the same condition (Shyamala et al., 2005). Second, water extract was prepared by means of a Percolator. Leaf samples were extracted with distilled water in a Percolator apparatus until the extracted water became colorless. Extracts were filtered and evaporated to dryness in vacuum. The crude extract was weighted and kept in a closed dark glass bottle and stored at 0-4°C until use.

In vitro Antimicrobial activity test: Disk Diffusion Test

The aqueous extracts were tested against *Staphylococcus aureus* ATCC 25913, *Escherichia coli* ATCC 8739 and *bacillus cereus* ATCC25730. The microorganisms were cultured in BHI (Brain Heart Infusion) for 18 hrs at 37◦C, and resuspended in 0, 5 Mac Farland Standard (5 x 10^8 CFU/mL) and inoculated directly in boards with Mueller-Hinton Agar (Merck). After the inoculation of each microorganism, the diffusion method was used, putting 10 µL of essential oil on paper disks (6 mm of diameter) at 37^{o}C for 24 hrs, after which

time the halos of inhibition were measured. Micro dilution method extracts were diluted by using serial micro dilution method with Mueller Hinton Broth culture medium at a final concentration range from 512 to 0.25%. Each and every extracts was assayed for antibacterial activity in triplicate. Before conducting experiments all the conditions were standardized to determine MIC and MBC values *in vitro* (Stefanini et al., 2001).

In vitro antioxidant activity: DPPH assay

The hydrogen atom or electron donation abilities of the corresponding extracts and some pure compounds were measured from the bleaching of the purple-colored methanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH). This spectrophotometer assay uses stable radical DPPH (Sigma, Aldrich) as the reagent (Cuendet et al., 1997). Briefly, 50 µL of the extracts (various concentrations) were added to 5 ml of the DPPH solution (0.004% methanol solution). After 30 min incubation at room temperature, the absorbance was read against pure methanol at 517 nm. The radical-scavenging activities of the samples were calculated as percentage of inhibition according to the following equation: % DPPH radical scavenging = [(control absorbance (blank) -sample absorbance)/ (control absorbance)] ×100

Extract concentration providing 50% inhibition (IC50) was calculated from the plot of inhibition percentage against extract concentration using PHARM/PCS-version 4. All tests were done in triplicate. Values (mean ± SD) of the extracts were compared with those of BHT using student's t-test. A p-value less than 0.05 were statistically considered significant (Gulluce et al., 2007).

β-Carotene linoleic acid assay: Antioxidant capacity was determined by measuring the inhibition of volatile organic compounds and the conjugated dienehydroperoxides arose from linoleic acid oxidation method (Dapkevicius et al., 1998). In this regard, stock solution of β-carotene linoleic acid mixture was prepared as follows: 0.5 mg β-carotene (Merck, K15555836) was dissolved in1 ml of chloroform (HPLC grade) and then 25 µl linoleic acid (Sigma, L1376-500 mg) and 200 mg Tween 40 (Merck, 822185) were added. After the evaporation of chloroform, 100 ml of oxygen saturated distilled water was added with vigorous shaking. Then, 2500 µl aliqouts were dispensed into the test tubes, 350 µl of the extract (2 g/L) was added and the emulsion system incubated for 48 hrs at room temperature. The same procedure was performed for both BHT (as positive control) and blank. In turn, absorbance spectra of the mixtures were obtained at 490 nm. Afterward, Anti oxidative capacities of the extracts were compared with those of BHT and blank. Further, all inhibition percentages were compared using with 95% confident interval (Oke et al., 2009).

Statistical analysis

Each experiment, from sample preparation to analysis, was repeated in triplicate, and the data were then analyzed by Excel software program version 2010.

Results and Discussion

Quercus infectoria aqueous extracts are shown to exhibit widespread antimicrobial activity. Data for *Quercus infectoria* aqueous extracts susceptibility testing by broth micro dilution are shown in Table 1. Gram positive bacterial strains studied were inhibited by galls of *Quercus infectoria* extracts, with same degrees of inhibition. The MIC value of *Quercus infectoria* aqueous extracts were as follows: *Staphylococcus aureus*, and *Bacillus cereus* obtained MIC300 µg/ml. The aqueous extracts of the galls of *Quercus infectoria* showed antimicrobial activity against

gram positive bacterial strains, used in this study (*Staphylococcus aureus*, with an inhibitory zone of 18 mm, and *Bacillus cereus*, with inhibitory zones of 18 mm). Our results showed that higher concentration of *Quercus*

Table 1. Minimum inhibitory concentration (MIC) (µg / mL) extract of oak gall

Bacteria	ATCC	Gram (- / +)	Aqueous extract	Ethanol extract	Methanol extract
Staphylococcus aureus	29737	+	300	600	1200
Bacillus subtilis	12711	+	600	1200	1200
Escherichia coli	873	-	4800	9600	9600
Pseudomonas aeruginosa	1074	-	1200	2400	2400
Klebsiella pneumoniae	10031	-	2400	4800	4800
Yersinia enterocolitica	1151	-	1200	2400	2400

Table 2. Minimum bactericidal concentration (MBC) (µg / mL) extract of oak gall

Bacteria	ATCC	Gram (- / +)	Aqueous extract	Ethanol extract	Methanol extract
Staphylococcus aureus	29737	+	600	1200	2400
Bacillus subtilis	12711	+	1200	4800	2400
Escherichia coli	873	-	9600	4800	> 9600
Pseudomonas aeruginosa	1074	-	2400	9600	4800
Klebsiella pneumoniae	10031	-	4800	9600	> 9600
Yersinia enterocolitica	1151	-	2400	4800	4800

Table 3. Comparison of the mean values of total phenolic compounds (mg Tannic acid/gm of extract) phenolic extracts from different solvents

Species	Solvent		
	Methanol 80%	Ethanol 70%	Aqueous
Andricus moreae	216 + 0/44[a]	187 + 0/77[b]	119 + 0/11[c]

Dissimilar letters in each row indicate significant differences at the 5% level

Fig. 1. The effect of Gall (*Andricus moreae*) and positive control BHT in scavenging free radicals DPPH °

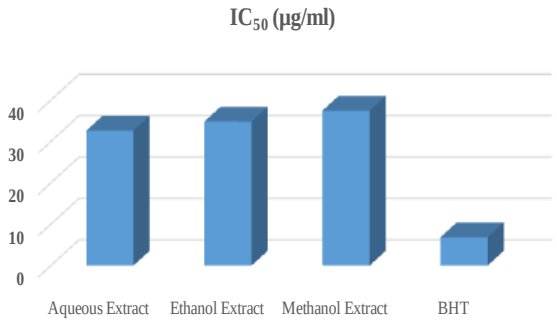
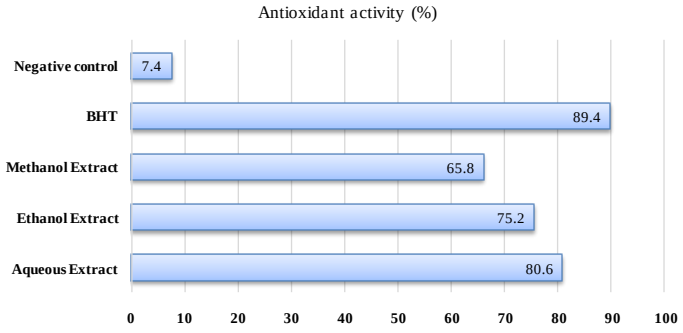


Fig. 2. Comparison with BHT antioxidant activity of the extracts, beta-carotene bleaching method, Concentration in 2 g/l ethanol



infectoria aqueous extracts increased its antibacterial effect. Antibacterial susceptibility was evaluated using classical microbiological techniques with disk diffusion, MIC and MBC determination (Tables 1, 2, 3).

In the present study, we also demonstrate the antioxidant effect of the extract of *Quercus infectoria* galls. The extract exhibited a remarkable antioxidant activity (LC50 = 30.15 µg/ml). Preliminary phytochemical investigation revealed the presence of phenolic compounds and flavanoids which have been reported to be associated with anti-oxidative action in biological systems acting as scavengers of singlet oxygen and free radicals (Halliwell, 1990). In summary, we conclude that most of the results of this study are in good agreement with the traditional uses of the investigated plant. All the extracts showed significant antibacterial and antioxidant activity. It was postulated that an increase in the antibacterial activity of pure compounds occurred when they are combined with antioxidants. Therefore, we consider that if both antibacterial and antioxidant compounds exist in the extracts, they could interact and enhance the antibacterial activity. Vermani et al. reported good antibacterial activity of Quercus methanolic extract against dental pathogens.

In this study, they showed which the gall extract better results than both the plants. The main constituents found in the galls of *Q. infectoria* are tannin (50-70%), gallic acid and ellagic acid (Vermani, 2009). Voravuthikunchai et al. reported good anti-bacterial activity of *Quercus infectoria* semipurified fractions of *Q. infectoria* against enterohemorrhagic *E. coli* O157:H7. (Voravuthikunchai and Suwalak, 2008). Basri et al. (2011) reported good anti-bacterial activity methanol and acetone extracts of *Q. infectoria* galls against oral pathogens. In this study, they showed that both extracts exhibited similar antibacterial activity against oral pathogens. Dokhaharani et al. reported good anti-bacterial activity water extracts of *Q. infectoria* galls against multi-drug-resistant gram negative bacteria. In this study, they showed that both extracts exhibited similar antibacterial activity against multi-drug-resistant gram negative bacteria (Dokhaharani, 2013). Baseri and Fan reported good anti-bacterial activity methanol and acetone extracts *Q. infectoria* galls against *S. aureus*, *S. epidermidis*, *B. subtilis*, *S. typhimurium* and *P. aeruginosa*. In this study, they showed that aqueous and acetone extracts displayed similarities in their anti-microbial activity on the bacterial species (Fan et al., 2014). Plant compounds is largely dependent on the type and location of grow and soil type and weather and type of solvent used in the extraction procedure. Traditional healers use primarily water as the solvent (Larcher, 2003). Belofsky et al. (2006) showed an increment in the antimicrobial activity of pure compounds when they are combined with antioxidants. Therefore, we consider that if both antimicrobial and antioxidant compounds exist in the extracts, they could interact and enhance the antimicrobial activity. The bioassay-guided fractionation of these extracts in order to isolate and identify the compounds responsible for each of these activities, followed by a study of their interaction, is highly desirable.

Conclusion

In this study, the antimicrobial and antioxidant activity of extracts of *Quercus infectoria* galls were performance against food-borne pathogenic bacteria. The extracts may be effective in other gram-positive and gram-negative bacteria. Practical application of various extracts and increases shelf life for food use. Therefore, it can be good alternative and satisfactory artificial preservatives used in the food industry today.

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