



## In Vitro Antimicrobial Activity of the Alcoholic Extract of *Quercus brantii* subsp. *persica*



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### ABSTRACT

**Background:** Lately, consumption of natural preservatives to improve food products' shelf life has been the center of attention. Due to the approved health benefits of the oak fruit, its application in food industry as a preservative seems satisfying.

**Methods:** Antimicrobial activity of the alcoholic extract of the oak fruit in concentrations of 200, 400, 600 and 800 mg/mL was investigated on *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumoniae*, and *Saccharomyces cerevisiae* with well diffusion technique. In order to detect the minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC), macro-dilution broth and subculturing on solid culture media techniques were used.

**Results:** The most growth prevention and inhibitory effect was observed in *S. aureus*, *S. cerevisiae*, *K. pneumoniae*, *S. typhi*, and *E. coli*, respectively. The most and the least antimicrobial activity of the oak fruit extract were observed in *S. aureus* (MIC = 0.15 mg/mL and MBC = 0.313 mg/mL) and *E. coli* (MIC=2.5 mg/mL and MBC=5 mg/mL), respectively.

**Conclusion:** The alcoholic extract of Iranian oak fruit had antimicrobial activity, and its impact was more profound on gram-positive bacteria such as *S. aureus* and *S. cerevisiae* than gram-negative ones namely *E. coli*, *K. pneumoniae*, and *S. typhi*.

## 1. Introduction

In recent years, due to the increased demand for healthy food, natural preservatives consumption has increased. Not only do they lack any side effects; but also, they enhance the shelf life of food products efficiently. Various plant species are found in Iran many of which contain industrial and pharmaceutical usage. In food industry, they are normally used as preservatives, antioxidant and flavoring agents. [1]. Oak forests have been distributed throughout Iran from west, southwest, and north to northwest, and several varieties have been detected in these regions. Each oak tree produces at least 15 kg of oak fruit in its fruiting years and thousands

of tons of oak fruits are produced annually. Unfortunately, the majority of them remain useless and are wasted in forests and only a small proportion is used to feed animals or produce tannins [2]. Iranian oaks (*Quercus persica*) are about twenty-meter-high trees with bulky crowns from Fagaceae family. Their leaves are smooth, dented with rounded teeth (crenate) and stellate trichomes on the upper surface of the leaves and yellow-colored ones on the lower surface. The oak fruit is a quasi-oval seed enclosed in a conical white velvet cup [3].

The oak fruit which is called acorn is placed in a cup named Gland. Acorn contains various amounts of oil, sugars, and starch. In addition, it is rich in bioactive chemicals namely



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gallic acid, tannins, ellagic acid, and hexahydroxydiphenic derivatives all of which possess antioxidant activity [4].

Anti-inflammatory, antibacterial, and antiviral properties of some oak species such as *Q. Persica*, evergreen oak and Darmaz oak have been reported by several researchers [4-7].

Tannins are among important antimicrobial agents in oak fruit which are categorized into condensed and hydrolysable tannins. Catechin and epicatechin belong to the former group, while gallic acid and ellagic acid belong to the latter [6]. The antimicrobial effect of tannins can be related to the inhibition of microbial binding and cell covering carrier proteins and enzymes deactivation. It seems that the oak antimicrobial activity is relative to the amount of tannins in the plant extract [7]. Due to the existence of tannins and other phenolic compounds in the oak fruit; we can expect the oak fruit to reduce microbial load and ensure food product hygiene.

Previous studies have investigated the antimicrobial effect of the oak fruit and the other parts of the tree [4, 5, 8 - 23]. However, this study investigated this effect in various concentrations using two different methods for a broader range of microorganisms (gram-positive, gram-negative, and yeast). It is noteworthy that due to the higher antimicrobial effect of the oak fruit in comparison with other parts of the tree (trunk, fruit shell, and leaves) [4], the oak fruit was chosen for this study. Thus, this study aimed to investigate the antimicrobial effect of the alcoholic extract of Iranian oak fruit (*Q. persica*) against *E. coli*, *S. aureus*, *S. typhi*, *K. pneumoniae*, and *S. cerevisiae* via well diffusion and macrodilution broth techniques.

## 2. Materials and Methods

### 2.1. Materials

Acorn was purchased from local markets, Tehran, Iran and was identified as *Quercus brantii* subsp. *persica* by the herbarium group, agriculture department of Shiraz University, Shiraz, Iran. Fresh fruits were collected and instantly dried under suitable conditions (at room temperature for three days). While drying, they were prevented from exposure to light and moisture to avoid any possible chemical reaction that alters components' chemical structure. Research microorganisms were *Salmonella typhi* (PTCC 1709), *E. coli* (ATCC 19118), *Saccharomyces cerevisiae* (PTCC 5074), *Staphylococcus aureus* (ATCC 6538), and *Klebsiella pneumoniae* (ATCC 700603) which were provided lyophilized by Zist Kavosh Iranian center (Tehran, Iran). All medium cultures and chemicals were bought from Merck Co., Germany.

### 2.2. The Oak Extract Preparation

The seed coat was removed initially and the fruit powder was prepared by an electric mill through several steps and

was held in a refrigerator until use. To produce the extract, we removed fat by Soxhlet method. In this technique, 10 g of the fruit powder was mixed with 200 mL N-hexane to remove any fat and other oleoresins for 4-5 h. Then, the sample powder was dried in an oven (Memmert, Germany) at 100°C. In order to extract, the dried powder was kept in ethanol for 20 h and the extract was separated by a vacuum rotary (EIKA, Germany) at 80 °C and 1-atmosphere pressure [24].

### 2.3. Clarification of the effective Concentrations of the Oak Fruit Through Well Diffusion Method

Standard methods revived the bacteria. In order to prepare a microbial suspension, some colonies were transferred from a 24-h fresh culture media to sterilized distilled water and turbidity of 0.5 McFarland (equal to  $1.5 \times 10^8$  CFU/ml) was created and its absorption was measured at 620 nm with a spectrometer (EIKA, Hewlett-Packard, Germany). Subsequently, this suspension was diluted 100 times to achieve a turbidity of  $1.5 \times 10^6$  CFU/mL using the well diffusion method, and homogeneous cultivation of  $1.5 \times 10^6$  CFU/mL suspension was prepared in plates. Then, wells were placed at 2.5 cm intervals and 0.1 mL of the oak fruit extract was added with various concentrations ranging from 200 to 800 mg/mL. Plates containing nicin, tetracycline, and gentamicin were used as positive control for *S. cerevisiae*, *S. aureus*, and *S. typhi*, respectively, and sterile distilled water was used as the negative control. Having incubated for 24 h in an incubator (Memmert, Germany) at 37 °C, the diameter of growth prevention was measured and reported by the Cm scale [16]. To verify results, experiments were repeated 3 times for each bacterial sample.

### 2.4. MIC and MBC Analysis of the Oak Fruit Extract against Certain Bacteria in Macrodilution broth Method

For measuring MIC for the oak fruit extract, a series of 12 test tubes were used. 200 mg/mL of the extract was diluted in 10 tubes containing nutrient broth. The 11th tube was used as the positive control (medium culture and extract), and the 12th tube was considered as the negative control (microbial suspension and medium culture). Extract dilution was conducted for each bacterium, separately (*E. coli*, *S. aureus*, *S. typhi*, *K. pneumoniae*, *S. cerevisiae*). All test tubes were held at 37 °C for 24 h. After this time, tubes were analyzed from turbidity as a result of inoculated bacterial growth. Their absorption was consequently measured at 620 nm, and finally, the minimum concentration of the extract in which no change in optical density was observed was considered as MIC. All test tubes without bacterial growth were sampled and surface cultivation was conducted on nutrient agar. Plates were then incubated for 24 h to monitor bacterial growth. Tubes with a 99% reduction in bacterial growth were chosen as MBC [25].

### 2.5. Data Analysis

Data analysis was conducted by a completely randomized factorial design. Duncan test was used to compare treatments at 95% significance level. SAS 9.2 (SAS Institute, Inc., Cary, NC) was used to analyze data by 3 repetitions.

## 3. Results and Discussion

Effective concentrations of the oak extract were initially measured using well diffusion technique (Table 1). Bacterial inhibition zone diameter at 200 mg/mL was significantly lower than other concentrations, and others showed no noticeable difference ( $P < 0.05$ ). In addition, no significant difference was observed in bacterial inhibition zone diameter in *S. cerevisiae* at 200, 400, 600, and 800 mg/mL.

According to the scientific description of MIC and MBC, the least concentration preventing the growth and the least concentration leading to bacterial death are considered as MIC and MBC, respectively [9]. MIC and MBC were subsequently investigated for the oak fruit alcoholic extract. Based on Table 2, the least MIC and MBC were observed in *S. aureus* at 0.156 and 0.313 mg/mL, respectively, and the most were measured in *E. coli* at 2.5 and 5 mg/mL, respectively. MIC and MBC were measured 1.25 and 2.5 mg/mL in *S. typhi* and coliform, respectively.

Results of the present study revealed that the oak fruit alcoholic extract expresses an antimicrobial effect on bacteria and prevents their growth. In all bacteria, bacterial inhibition zone diameter was smaller at 200 mg/mL than other concentrations. The highest and lowest inhibition zone diameters were seen in *S. aureus* and *E. coli*. It can be concluded that in the well diffusion method, when the concentration of the extract increased, the antimicrobial activity increased, too (except for *S. cerevisiae*), and the antibacterial activity of the extract could be attributed to its bioactive compounds. The effectiveness trend of the oak fruit extract reveals that this material possesses a certain antimicrobial effect which increases relatively with concentration or in other words, with the increase of its active compounds.

However, this effect is more predominant in *S. aureus* (a gram-positive bacterium) than in gram-negative ones such as *E. coli*, coliform, and *S. typhi* which may be related to an

outer layer that contains hydrophilic lipopolysaccharides that form a barrier against hydrophobic materials and macromolecules into lipopolysaccharide layer. Subsequently, gram-negative bacteria represent higher resistance toward hydrophobic antimicrobial agents [26].

The oak fruit antimicrobial effect is due to tannins existing in the extract. Tannins are flavonoids with various features, namely antimicrobial activity [4, 27]. Its major effect is relative to its hygroscopic effect and protein sedimentation. It may be possible that their antimicrobial activity is because of their capability of microbial adhesion and enzyme or cell membrane transport protein deactivation. Tannins can be toxic to bacteria, yeast, filamentous fungi, or viruses. They can prohibit proteins from reaching the microorganisms and or play a role through ferrous trapping mechanism, specific bonding to vital proteins, namely enzymes and hydrogen bonds [10, 15, 17, 28]. Phenolic compounds chelating capacity for transporting metals, namely iron, and copper, can reduce the reactivity of metallic ions by forming a neutral metal-ligand complex. As a result, the bioavailability of these metals for bacterial growth declines [14]. Antimicrobial activities in almost all microbial essences regularly occur through interaction with bacterial cell membrane processes [29]. Gram-positive microorganisms usually are more sensitive than gram-negative bacteria towards plant extracts [30]. Oussalah *et al.* (2007) reported that *S. aureus* is more sensitive than *S. typhi* and *E. coli* towards essential oils and explained this with the fact that *S. aureus* possesses a single-layer membrane [31].

In agreement with the results of this study, several researchers have admitted the concentration-dependent antimicrobial effects of oak in different parts of the tree, which is as follow:

Behzadi and Khosravi (2001) and Khosravi and Behzadi (2006) investigated the antimicrobial effect of the oak seed hull and the oak extract on bacterial inhibition zone diameter for some bacteria, respectively. They concluded that the extract effect on *E. coli* and *Proteus mirabilis* increased as a consequence of increased concentration. On the other hand, this effect had no relation with concentration on *Shigella* and *Salmonella* [10, 11]. Basri and Fan (2005) reported that the acorn extracts of galls of *Q. infectoria* are more effective against gram-positive bacteria than gram-negative ones [12]. Hayouni *et al.* (2007) announced that gram-positive bacteria,

**Table 1:** Effect of different concentrations of the oak fruit extract on growth of the studied microbial samples\*

Concentration of extract	Microorganism				
	<i>E. coli</i>	<i>S. aureus</i>	<i>S. typhi</i>	<i>S. cerevisiae</i>	<i>K. pneumoniae</i>
200 mg/mL	2.72±0.403 <sup>b</sup>	3.73±0.341 <sup>b</sup>	3.23±0.326 <sup>c</sup>	3.55±0.266 <sup>a</sup>	3.1±0.362 <sup>b</sup>
400 mg/mL	3.09±0.403 <sup>a</sup>	3.94±0.341 <sup>a</sup>	3.52±0.326 <sup>b</sup>	3.57±0.266 <sup>a</sup>	3.32±0.326 <sup>a</sup>
600 mg/mL	3.25±0.403 <sup>a</sup>	3.96±0.341 <sup>a</sup>	3.7±0.326 <sup>a</sup>	3.69±0.266 <sup>a</sup>	3.43±0.326 <sup>a</sup>
800 mg/mL	3.37±0.403 <sup>a</sup>	3.98±0.341 <sup>a</sup>	3.75±0.326 <sup>a</sup>	3.75±0.266 <sup>a</sup>	3.55±0.326 <sup>a</sup>

\*Zone of inhibition is in centimeters. Well diffusion method is used. Data are presented as Mean ± Standard deviation.

a,b In each column, the averages with different Latin letters indicate a significant difference ( $P < 0.05$ ).

**Table 2:** MIC and MBC (mg/ml) of the oak ethanolic extract and some antibiotics against strains of studied microorganisms

Microorganism	MIC	MBC	nisin	tetracycline	Gentamicin
<i>E. coli</i>	2.5	5	----	----	0.032
<i>S. aureus</i>	0.156	0.313	----	0.032	----
<i>S. typhi</i>	1.25	2.5	----	----	0.032
<i>K. pneumoniae</i>	1.25	2.5	----	----	0.032
<i>S. cerevisiae</i>	0.625	1.25	0.016	----	----

---disuse of antibiotics is to determine the minimum inhibitory concentration.

namely *S. aureus* were more simply controlled by Tunisian *Quercus coccifera* L. and *Juniperus phoenicea* L. fruit extracts than gram-negative bacteria such as *E. coli*. Moreover, they presented the plausible explanation of lipopolysaccharides existing in cell membrane of gram-negative bacteria. This membrane makes the bacteria tolerant to external factors such as hydrophilic dyes, antibiotics, and detergents [13]. Safari *et al.* (2009) investigated the antimicrobial effect of the ethanolic and methanolic extract of *Q. brantii* on intestinal pathogenic bacteria [14]. Taran *et al.* (2010) claimed that *Q. persica* hydro-alcoholic and etheric extract inhibited fungus, gram-positive, and gram-negative bacteria growth and reported that the existence of an extra layer in gram-negative cell membrane reduces material infusion to cells. Therefore gram-positive bacteria are more sensitive to the hydro-alcoholic and etheric extract than gram-negative ones [9]. Ebrahimi *et al.* (2012) concluded that with an increase in the concentration of metabolic extract of the oak fruit, bacterial inhibition zone diameter increased and did the antimicrobial effect. Additionally, gram-negative bacteria showed the highest resistance compared to gram-positive ones [4]. Sharif *et al.* (2012) reported that the hydro-alcoholic extract of *Q. infectoria* gall prevented *Saprolegina* fungi growth, and its antimicrobial effect increased as a consequence of the extract concentration enhancement (25-300 mg/ml) [15]. Sadeghian *et al.* (2012) reported the antimicrobial activity in several parts of *Q. brantii* against gastrointestinal bacterial pathogens [8]. Sefidgar *et al.* (2015) investigated the antimicrobial effect of the aqueous and ethanolic extracts of the oak fruit against *E. coli*, *S. aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. They reported that the alcoholic extract had a stronger inhibitory effect. *E. coli* and *P. aeruginosa* expressed the least resistance, and *S. aureus* showed the highest resistance towards both extracts [16]. Borjian and Brujeni (2016) reported that the hydro-alcoholic extract of acorn has inhibitory and toxic effects on *Listeria monocytogenes* and *Enterococcus faecalis* [17]. Midi and Sharifi (2017) emphasized that the methanolic extract of *Q. brantii* has antimicrobial and anti-filming properties against *P. aeruginosa* [18]. On the other hand, Shahi *et al.* (2017) claimed that the methanolic extract of *Q. brantii* had no noticeable effect on *P. aeruginosa* strains [19]. Bahar *et al.* (2017) found that *Q. brantii* subsp. *persica* aqueous extract was active against *S. epidermidis*, *S. aureus*, *P. aeruginosa*, and *E. coli* and had anti-biofilm features [20].

Fatehi *et al.* (2018) proved the antimicrobial effect of the methanolic and aqueous extracts of Mazuj and Ghalghaf galls on *P. aeruginosa* while the methanolic extract proved to be stronger than the aqueous one, and anti-biofilm properties had a positive correlation with concentration [21]. Serif *et al.* (2018) mentioned that antimicrobial activity of the oak hydro-alcoholic extract is positively related to concentration in *S. aureus*, *E.coli* and *S. epidermidis* [22]. Alebrahim-Dehkordy *et al.* (2019) reported the inhibitory effect of the oak hydro-alcoholic extract in *S. aureus* and *Enterococcus faecalis* and found *S. aureus* more sensitive [23].

#### 4. Conclusion

This study confirmed the antimicrobial potential of the ethanolic extract of *Q. Persia*. Antimicrobial activities of the extract varied related to the studied bacteria. The data showed that the gram-positive bacterium *S. aureus* was more sensitive to the extract than gram-negative bacteria (*E. Coli*, *S. typhi*, and *Klebsiella pneumoniae*). In this study, *S. aureus* was the most sensitive to the ethanolic extract of *Q. Persia*, while *E. coli* was the most resistant. The antibacterial activity of the extract against the tested bacteria were increased when used in higher concentrations. Based on our findings, this extract can be used as a natural preservative in food products to increase their shelf life.

#### Authors' Contributions

**Marjan Zarei:** Formal analysis; Investigation, Visualization, Software; Writing-original draft. **Vajiheh Fadaei:** Conceptualization; Data curation; Investigation ; Methodology ; Project administration; Resources; Supervision; Validation; Writing-review and editing. **Mahta Mirzaei:** Conceptualization; Investigation; Methodology; Supervision; Validation; Writing-review and editing.

#### Conflicts of Interest

The Authors declare that there is no conflict of interest.

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