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Antimicrobial Effects of *Quercus Brantii* Fruits on Bacterial Pathogens

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ABSTRACT

Background: In recent years, a number of antibiotics have lost their effectiveness due to the development of resistant strains, mostly through the expression of resistance genes.
Objectives: The aim of the present study was to investigate the antimicrobial property of *Quercus brantii* fruits and compare its effects with some current antibiotics.
Materials and Methods: The antimicrobial activities of an ethanol extract of *Q. brantii* (Oak) fruits (brown cortex: B.C and white core: W.C) were tested in vitro against eight reference strains of enteric pathogenic bacteria. The antimicrobial activities of the extracts were examined based on the disc diffusion method. The results were evaluated as inhibition zones around the discs impregnated with B.C and W.C extracts at different concentrations (2 to 10 %).
Results: The antibacterial effect of the B.C ethanolic extract on *Escherichia coli* was significant and had a concentration-related effect, although there was no significant effect found on *Helicobacter pylori*. The W.C ethanolic extract has a high antimicrobial effect on *Streptococcus pyogenes*; at the same time significant antibacterial activity occurred against *H pylori*. Comparisons between the antimicrobial activities of these extracts (B.C and W.C) and standard antibiotics; gentamicin, colistin, and methicillin, showed that in the most commonly tested bacteria the antibacterial activity of these extracts was even greater than with the antibiotics. Analysis of the extracts components by gas chromatography, showed that tannins and phenolic compounds could be responsible for these antimicrobial activities.
Conclusions: The results of this study showed that different parts of *Q. brantii* have antimicrobial activity against gastrointestinal bacterial pathogens. These antimicrobial activities, in almost all cases, were greater than with standard antibiotics.

▸ *Implication for health policy/practice/research/medical education:*
The fruit of *Q. brantii* are used in decoction or as powder to treat acute diarrhea and inflammation in traditional medicin.

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1. Background

Gastrointestinal problems produced by bacteria are widespread throughout the world. The treatment of these infections is mainly based on the use of antibiotics (1). In recent years, a number of antibiotics have lost their effectiveness due to the development of resistant strains, mostly through the expression of resistance genes (2, 3). In addition to this problem, antibiotics are sometimes associated with adverse effects including; hypersensitivity, immune-suppression and allergic reactions (4). Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from a variety of sources, such as medicinal plants (5). Oak (*Quercus)* is a predominant plant genus in northern and central Iran and it is comprised of many species. *Quercus,* which grows in the central forest areas of the country, is one of the most important genus with 45 species; the predominant species being *Quercus brantii* (5). The fruits of this

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plant are used in a decoction or as powder to treat acute diarrhea and inflammation in traditional medicine (5, 6).

2. Objectives

The aim of the present study was to investigate the antimicrobial property of *Q. brantii* fruits and compare its effects with some current antibiotics.

3. Materials and Methods

3.1. Plant Material

The fruits of the plant were collected from the Fars area (south of Iran) in April 2011. The taxonomic identification of the plant material was confirmed by a plant taxonomist in the botany laboratory of the Shahid Bahonar University of Kerman. The fruits of the *Quercus* were crushed and divided into two parts: brown cortex (known as B.C) and white core (known as W.C).

3.2. Preparation of Discs Containing Extracts

The collected plant material was shade dried and powdered using an electric blender. One gram of this powder was extracted using 10 ml of ethanol distilled water (70 %) for 24 hours. The ethanolic extract was filter through Watman filter No. 4 and concentrated by centrifuge (3000 rpm for 5 min). This process was repeated three times.

Different concentrations of 2, 4, 6, 8 and 10 % were prepared from the ethanolic extract of W.C and B.C plant material separately. The concentrations were incorporated into sterile blank paper discs (Padtan Teb Inc, Tehran, Iran) and were dried at 37 °C. The paper discs were weighed carefully to confirm the exact amount of the extract that was incorporated (when compared to the preweighed blank discs) (8, 9).

3.3. Bacterial Strains

Eight strains of bacteria from the Persian Type Culture Collection (PTCC) were tested: *Esherichia* coli (PTCC 1112), *Pseudomonas aeruginosa* (PTCC 2381), *Proteus mirabils* (PTCC 3412), *Staphylococcus aureus* (PTCC 1311), *Streptococcus. pyogenes* (PTCC 3012), *Bacillus cerus* (PTCC 1812), *Klebsiella pneumoniae* (PTCC 4211) and *Helicobacter pylori* (PTCC 5211). A few colonies from the overnight culture of the Eosin Methylene Blue (EMB, Merck, Germany) agar were transferred into a Nutrient Broth (NB, Merck, Germany) medium. The broth was incubated at 37°C for 12 hours and the turbidity of the suspension was adjusted to that of 0.5 McFarland. The standard suspension was used for the qualitative antimicrobial assay (7).

3.4. Determination of Antimicrobial Activity

The antimicrobial activity of the plant sample ethanolic extracts (B.C and W.C) was evaluated by the paper disc diffusion method. One milliliter of 0.5 McFarland bacterial suspension was added to each plate containing Muller-Hinton Agar (MHA, Merck) by a sterile cotton swab and allowed to remain in contact with the plate for 1 min. Paper discs with different concentrations of B.C and W.C ethanolic extract were placed on these cultures. The plates remained at room temperature for 1 hour in order to diffuse the extract across the surface, and then they were incubated at 37°C for 24 hours. The inhibition zone around each disc was measured in millimeters and the assay carried out three times for each extract. Three different discs containing standard antibiotics (gentamicin, colistin, methicillin, Sigma, USA) served as positive controls and these were used to compare the antimicrobial activity of the ethanolic extracts. Discs impregnated with 70 % ethanol were also included as a negative control (8).

3.5. GC and GC-MS Analysis

Analysis by gas chromatography (GC) was carried out using a Hewlett-Packard 58900 chromatograph equipped with FID detector and a HP-1 column (60 m x 0.25 mm, fused silica and film thickness of stationary phase 0.25 cm). GC-MS (gas chromatography/mass spectroscopy) analysis was carried out on a Hewlett-Packard 5793 coupled with a mass detector HP 6890 using a HP-1 column (55 m x 0.25 mm, film thickness 0.25 cm). The experimental condition was as follows: oven temperature programmed from 40°C (1 min) to 250°C (30 min) at the rate of 3°C/min (for GC-FID and GC-MS), injector and detector temperature were 320°C and 310°C, respectively, the carrier gas was helium (99.99 %) at a flow rate of 1 ml/min. The mass spectrometer was operated at 70 eV with a mass range of 40-350 atomic mass unit and scan time of 1s. The identification of the compounds was based on comparison of their retention indices on the mass spectra with those of authentic samples and with the NIST (National Institute Technology Masochist) MS library. The identification was also confirmed by comparison of the retention indices with data in the literature (12).

4. Results

In this study the antimicrobial activity of an ethanolic extract of different fruit parts of *Q. brantii* (B.C and W.C) were evaluated against eight bacterial pathogens. The results showed that these plant extracts (B.C and W.C) were effective against the test microorganisms. The highest antimicrobial activity of B.C ethanolic extract was observed on *E coli* (inhibition zone diameter about 28 mm), while the lowest activity of this extract was observed against *H. pylori* (without any inhibition zone) (*Table 1*). The antimicrobial effect of different concentrations of B.C ethanolic extract on bacterial pathogens showed that the antibacterial activity of B.C ethanolic extract against the tested bacteria was increased when used at higher concentrations. However the differences between the 2 % and 4 % concentrations were significant in comparison to the

Table 1. Antimicrobial Activity of *Q. brantii* B.C [a] Ethanolic Extract Against Bacterial Strains Tested by Disc Diffusion Method at Different Concentrations (Inhibition Zone mm)

	2 %	4 %	6 %	8 %	10 %	Negative Control, (Ethanol)
Esherichia coli, mm	15	23	25	27	28	0
Pseudomonas aeruginosa, mm	15	16	20	21	22	0
Proteus mirabils, mm	14	18	20	23	25	0
Staphylococcus aureus, mm	15	17	20	22	23	0
Streptococcus pyogenes, mm	14	18	20	21	23	0
Bacillus cerus, mm	15	17	18	20	21	0
Klebsiella pneumoniae, mm	16	18	18	19	21	0
Helicobacter pylori, mm	0	0	0	0	0	0

[a] Abbreviations: B.C, Brown Cortex

other concentrations (6 %, 8 % and 10 %) (*Table 1*). The W.C ethanolic extract was also effective against the test microorganisms. The highest antimicrobial activity of the W.C ethanolic extract was demonstrated against *S. pyogenes* (inhibition zone diameter approximately 28 mm), while the lowest activity of this extract was observed against *P. mirabilis* (inhibition zone approximately 17 mm) (*Table 2*). The W.C ethanolic extract had a high antimicrobial activity against *H. pylori* and this bacterium is the cause of a common stomach disorder; in contrast the B.C ethanolic extract did not have any antimicrobial activity on *H. pylori* (*Table 2*). When the concentrations of the W.C ethanolic extracts were increased, the antimicrobial activity increased too (*Table 2*). The results of a comparison between B.C and W.C ethanolic extracts with standard antibiotics showed that in the most commonly tested bacteria the antimicrobial activity of B.C and W.C ethanolic extracts were greater than the antibiotics, but there was some exceptions such as the antibacterial activity of colistin against *P. aeruginosa* and *S. aureus* which were similar to the B.C and W.C ethanolic extracts. Gentamicin had an antibacterial effect against *K. pneumonia* similar to the W.C ethanolic extract. The antibacterial activity of meticillin on *P. aeuroginosa* was more than for the B.C and W.C ethanolic extracts; however, the effect of this antibiotic on *B. cerus* was similar to the W.C ethanolic extract (*Table* 3). Also, some of the antibiotic resistant bacteria were observed in the standard antibiotics that were used (*Table 3*). The GC analysis of the B.C and W.C ethanolic extracts were carried out to determine the composition of these

Table 2. Antimicrobial Activity of *Q. brantii* W.C [a] Ethanolic Extract against Bacterial Strains [b]

	2 %	4 %	6 %	8 %	10 %	Negative Control, (Ethanol)
Esherichia coli, mm	15	18	18	20	21	0
Pseudomonas aeruginosa, mm	10	13	14	15	18	0
Proteus mirabils, mm	12	13	14	15	17	0
Staphylococcus aureus, mm	15	15	15	16	17	0
Streptococcus pyogenes, mm	10	17	23	26	28	0
Bacillus cerus, mm	10	13	16	18	20	0
Klebsiella pneumoniae, mm	12	14	16	16	17	0
Helicobacter pylori, mm	18	19	20	21	24	0

[a] Abbreviations: W.C, White Core

[b] Tested by Disc Diffusion Method at Different Concentrations (Inhibition Zone mm).

Table 3. Comparisons of Antimicrobial Activity of Q. brantii B.C [a] and W.C [a] Ethanolic Extracts with Standard Antibiotics (Inhibition Zone mm)

	BC [a]	WC [a]	GM [a]	CL [a]	ME [a]	Negative Control, (Ethanol)
Esherichia coli, mm	15	15	R [a]	10	10	0
Pseudomonas aeruginosa, mm	15	10	3	15	18	0
Proteus mirabils, mm	14	12	8	8	R [a]	0
Staphylococcus aureus, mm	15	15	R	16	5	0
Streptococcus pyogenes, mm	14	10	2	11	6	0
Bacillus cerus, mm	15	10	R [a]	R [a]	10	0
Klebsiella pneumoniae, mm	16	12	12	6	R [a]	0
Helicobacter pylori, mm	0	18	R [a]	R [a]	4	0

[a] Abbreviations: B.C, Brown Cortex; W.C, White Core; GM, Gentamicin; CL, Colistin; ME, Methicillin; R, Resistent

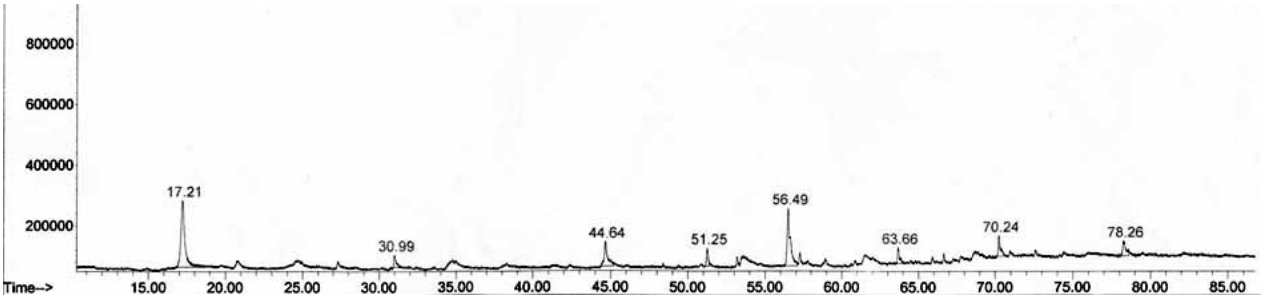

Figure 1. GC Analysis of *Q. brantii* Brown Cortex Ethanolic Extract

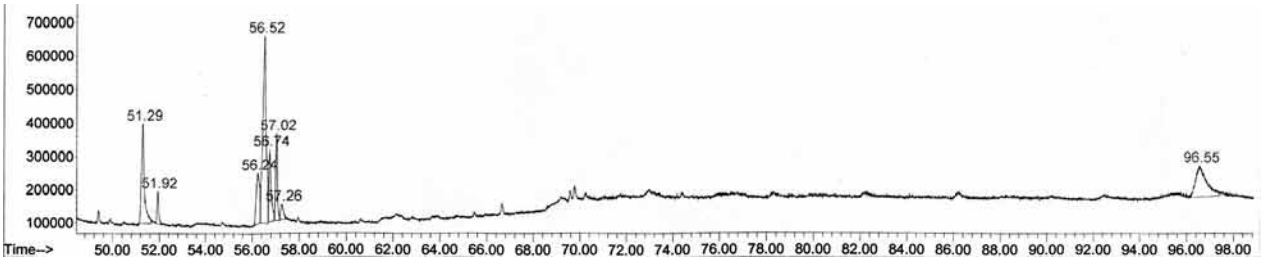

Figure 2. GC Analysis of *Q. brantii* White Core Ethanolic Extract

extracts. The results are shown in *Figure 1* and *Figure 2*. As can be seen in these figures, the major components of the B.C and W.C ethanolic extracts are; fatty acids, esters, tannins, alkaloid and phenolic compounds.

5. Discussion

Antibacterial resistance among bacterial pathogens, especially gastrointestinal pathogens is an important issue that has created a number of problems in the treatment of enteric diseases and necessitates the search for alternative drugs or natural anti-bacterial agents. Medical plants could be one approach because most of these are safe and have fewer side effects, but they are able to effect a wide range of antibiotic resistant microorganisms (9). The results of the present study showed that an ethanolic extract from the fruits of *Q. brantii* (B.C and W.C) inhibited the growth of various species of bacterial pathogens, especially enteric pathogens. The W.C ethanolic extract of *Q. brantii* showed significant effects on *H. pylori*. Stomach disorders caused by this bacterium are widespread throughout the world and an ethanolic extract could be useful for the treatment of this disease. Bahdor and Baserisalehi (2011) examined a methanolic extract of *Q castaneifolia* and reached a similar result to that reported in this study.

Present results are in agreement with the observations of other researchers such as; Gulluce *et al.* (2004) and Berahou *et al.* (2007) (10, 11). However, there are numerous ongoing research projects regarding the antimicrobial effects of other plants in general, most of which involved the *Labiatae* genus. Larrondo *et al.* (1995) in a similar survey using *Labiatae* plants concluded that the plants under investigation showed both antibacterial and antifungal activity (12). The results of a GC analysis revealed the component in the fruits of *Q. brantii* which is most likely to exhibit the antimicrobial activity. Tannin was the most abundant compound in the B.C and W.C ethanolic extracts. Tannins could be one of the components responsible for the antibacterial activity since it has been reported in other studies that tested different plants (13, 14). The major effect of this component is anti-diarrheal because of its water absorption and protein precipitation properties (15). Fatty acids and esters were other compounds that were isolated from B.C and W.C ethanolic extracts. These compounds are known to exhibit antibacterial activities (16-18). On the other hand, the hydrophobic character of these compounds can potentially impair cellular function and membrane integrity. The new aspect of this research is that for the first time the antibacterial activity of an ethanolic extract of *Q. brantii* was examined against *H. pylori* and the W.C ethanolic extract of *Q. brantii* showed a significant effect on *H. pylori*. This study was done in vitro without interfering with the body's physical factors (such as gastrointestinal movements) and chemical effects (stomach enzymes and acid, mucous, etc). However, the response in the body might be quite different and requires additional investigation of these natural factors. Further studies are also needed to determine the precise in vivo

antimicrobial activity of the plant extracts. The results of this study show that different parts of *Q. brantii* have antimicrobial activity against gastrointestinal bacterial pathogens. These antimicrobial activities in almost cases were greater than in the standard antibiotics.

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