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A Preliminary Study on the Antibacterial Activity of *Quercus brantii* Against Bacterial Pathogens, Particularly Enteric Pathogens

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Abstract: The antibacterial activity of *Q. brantii* fruits ethanolic and methanolic extracts were examined using agar disc diffusion method against eight bacteria (*Salmonella typhi*, *Proteus mirabilis*, *Shigella dysenteriae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Brucella melitensis*, *Bordetella bronchiseptica*, *Pseudomonas aeruginosa*). These extracts had inhibitory effect at various concentrations (0.5, 0.1, 0.2, 0.3 and 0.4 g mL^{-1}) against tested bacteria. The ethanolic extract had the highest activity (30 mm) against *Br. melitensis* and *B. bronchiseptica* while the lowest activity (7 mm) was demonstrated by the methanolic extract on *E. coli*. Studies on the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the methanolic extract on tested microorganisms showed that the highest MIC (20 mg mL^{-1}) and MBC (32 mg mL^{-1}) were demonstrated against *Sh. dysenteriae*, *B. bronchiseptica* and *P. mirabilis* had the highest MIC and MBC values (32 mg mL^{-1}) for the ethanolic extract.

Key words: Plant extract, *Quercus brantii*, antibacterial activity, pathogen

INTRODUCTION

Plants remain the most common source of antimicrobial agents. Their usage as traditional health remedies is the most popular for 80% of world population in Asia, Latin America and Africa and is reported to have minimal side effects (Doughari, 2006). Infectious diseases account for about half of the death in tropical countries (Khosravi and Behzadi, 2006). Besides, incidents of epidemics due to drug resistant microorganisms pose enormous public health concerns (Jensen *et al*., 1996; Burt and Reinders, 2003). Many studies indicates that in some plants there are many substances such as peptides, unsaturated long chain aldehydes, alkaloidal constituents, some essential oils, phenols and water, ethanol, chloroform, methanol and butanol soluble compounds (Alma *et al*., 2003; Klausmeyer *et al*., 2004). These plants then emerged as compounds with potentially significant therapeutic application against human pathogens, including bacteria, fungi or viruses (Holetz *et al*., 2002; Perez, 2003).

The antimicrobial compound from plants may inhibit microbial growth by different mechanisms (Zuraini *et al*., 2007). Oak plant is a predominant genus in Northern and Central parts of Iran and comprises many species (Khosravi and Behzadi, 2006). *Quercus brantii* widely grown in Izeh, Iran and the fruits of the plant have been locally known as Jaft. A decocted extract from *Q. brantii* fruits is used to treat acute diarrhea and inflammation in traditional medicine. Moreover, the decoction of *Quercus* could be also used for burns and cuts (Konig *et al*., 1994). The aim of the present study was to investigate the antimicrobial property of *Q. brantii* fruit and compare its effects with some current antibiotics.

MATERIALS AND METHODS

Collection and identification of plant materials: The plants used in this study were collected from Izeh in Khuzestan Province of Iran in 2007. The taxonomic identity of this plant was confirmed by us. Voucher specimens were deposited at the Department of Biology, Shahid Chamran University, Iran.

Preparation of extracts: The fruits of *Quercus* were shade dried and crushed into powder using electric blender. One gram of this powder was extracted using 10 mL of ethanol-distilled water (8:2 w/v), centrifugation (3000 rpm) for 15 min and then collecting the supernatants. This process was repeated three times. Finally the ethanol was removed through evaporation by incubating at room temperature (Seyyednejad *et al*., 2001; Moazedi *et al*., 2007). The methanolic extract was prepared following the method described by Okemo *et al*. (2001).

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Test bacteria: A total of 8 bacterial species were tested. *Salmonella typhi*, *Proteus mirabilis*, *Shigella dysenteriae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Brucella melitensis*, *Bordetella bronchiseptica*, *Pseudomonas aeruginosa*. These species were originally isolated from clinical materials collected from patients. They were identified using standard biochemical tests.

Determination of antimicrobial activity: Antimicrobial activity of the ethanolic and methanolic extracts of the plant sample was evaluated by the paper disc diffusion method (Sagdic and Ozcan, 2003). Stock culture of test bacteria were grown in TSB medium at 37°C for 22 h. Final cell concentrations were 10^8 cfu mL^{-1} with reference to the Mc Farland turbidometry (Burt and Reinders, 2003). One milliliter of this inoculum was added to each plate containing Mueller-Hinton agar (MHA, Oxoid) by sterile cotton swab and allowed to remain in contact for 1 min. Five concentrations of each extracts (0.5, 0.1, 0.2, 0.3 and 0.4 g mL^{-1}) were prepared. Sterile 6 mm filter paper discs (Hsieh *et al*., 2001) were placed on these cultures and immediately 50 μL volumes of the each concentration from the two mentioned extracts were added. The plates allowed to remain 1 h at room temperature in order to diffusing the extract across the surface and then were incubated at 37°C for 24 h. The inhibition zone around each disc was measured in millimeter and the assay was carried out three times for each extract. Discs containing different concentrations of three antibiotics (Novobiocin 30 mcg, Nafcillin 1 mcg, Colistin 10 mcg) served as positive controls. Discs impregnated with 80% ethanol were also included to test if it has inhibitory effect on the test bacteria in this study.

Determination of Minimum Inhibitory Concentrations (MIC): The Minimum Inhibitory Concentration (MIC) of the extracts was determined for the most sensitive bacterial species for three times. A 16 h culture was diluted with a sterile physiologic saline solution [PS; 0.85% (w/v) sodium chloride] with reference to the 0.5 McFarland standards to achieve inoculums of approximately 10^6 colony forming units (cfu) mL^{-1} (Burt and Reinders, 2003). A serial dilution was carried out to give final concentrations between 1 and 64 mg mL^{-1} from crude extract. The tubes were inoculated with 30 μL of the bacterial suspension mL^{-1} Muller Hinton broth, homogenized and incubated at 37°C. The Minimum Inhibitory Concentration (MIC) value was determined as the lowest concentration of the crude extract in the broth medium that inhibited the visible growth of the test microorganism.

Determination of Minimum Bactericidal Concentrations (MBC): To determine the MBC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes which did not show any growth and inoculated on sterile Muller-Hinton agar by streaking. Besides a Muller-Hinton agar was streaked with each of the test organisms respectively to serve as control. Plates inoculated at 37°C for 18-24 h. After incubation the highest dilution that yielded no single bacterial colony on a solid medium was taken as MBC.

RESULTS

The results showed that these plant extracts were effective against test organisms. The highest activity (inhibition zone diameter about 30 mm) was demonstrated by the ethanolic extract of *Q. brantii* Fruits against *Br. melitensis* and *B. bronchiseptica* while the lowest activity (inhibition zone diameter about 7 mm) was demonstrated by the methanolic extract against *E. coli* (Table 1).

On the other hand the ethanolic and methanolic extracts were not active against *K. pneumoniae* even in the highest concentration. However, the ethanolic extract showed inhibition action at minimal concentration (0.05 g mL^{-1}) used against *B. bronchiseptica*,

Table 1: Inhibition zone (mm)* of *Q. brantii* ethanolic and methanolic extracts at various concentrations on some bacteria

	Various concentrations of extracts												
	Ethanolic					Methanolic					Antibiotic discs		
Bacterial spp.	0.5	0.1	0.2	0.3	0.4	0.5	0.1	0.2	0.3	0.4	NF	NB	CL
Br. melitensis	15	20	25	27	30	16	20	22	25	26	R	25	R
E. coli	R	12	11	11	15	R	7	8	9	7	R	26	10
S. typhi	R	11	11	12	13	9	8	9	10	11	R	12	R
P. mirabilis	13	15	18	17	15	12	13	14	15	15	R	30	12
B. bronchiseptica	23	28	29	30	30	20	23	25	26	26	R	24	R
P. aeruginosa	8	13	12	14	15	9	11	11	12	13	R	R	14
Sh. dysenteriae	14	18	22	17	17	15	15	19	20	20	R	15	R
K. pneumoniae	R	11	R	R	R	R	R	R	R	R	R	21	14

R: Resistant, NF: Nafcillin 1 mcg, NB: Novobiocin 30 mcg, CL: Colistin 10 mcg, *Diameter of disc 6 mm

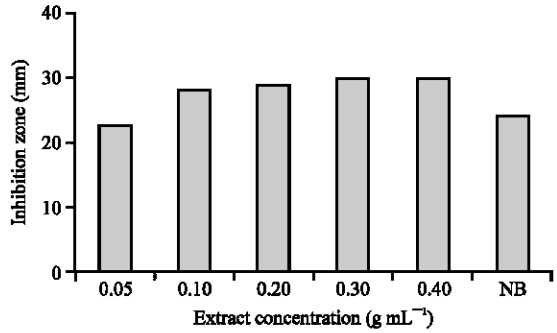

Fig. 1: Comparison of inhibition zone diameter produced by Novobiocin with different concentrations of ethanolic extract in disc diffusion method for *B. bronchiseptica*

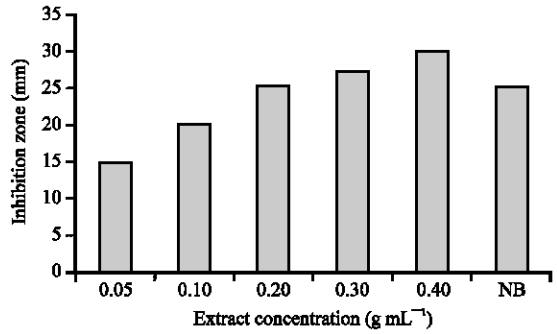

Fig. 2: Comparison of inhibition zone diameter produced by Novobiocin with different concentrations of ethanolic extract in disc diffusion method for *Br. melitensi*

P. aeruginosa, *P. mirabilis*, *Sh. dysenteriae* and *B. melitensis*. These results suggesting that antibacterial activity of *Q. brantii* ethanolic and methanolic extracts against tested bacteria were increased when used in higher concentrations. Also the methanolic extract generally showed lower activity against the test organisms compared to the ethanolic extract. In *Br. melitensis* and *B. bronchiseptica* the inhibition zone for Novobiocin compared to various concentrations of ethanolic extract was significant (Fig. 1, 2). For methanolic extract, the result showed that *Sh. dysenteriae* had the highest MIC (20 mg mL^{-1}), while the lowest MIC (16 mg mL^{-1}) and highest MBC (32 mg mL^{-1}) were shown by *B. bronchiseptica*. *P. mirabilis* had the highest MIC and MBC values (32 mg mL^{-1}) for the ethanolic extract. The MIC and MBC values for ethanolic extract were generally lower than methanolic extract against the test organisms. Alcohol impregnated discs containing 80% ethanol and methanol did not have a zone of inhibition due to the volatile nature of ethanol, so these were not considered as a factor that might affect the results (Table 2).

Table 2: Antibacterial activity (MIC[a] and MBC[b] in mg mL^{-1}) of the ethanolic and methanolic extracts from *Q. brantii* on some tested bacteria

	Methanolic extract (mg mL^{-1})		Ethanolic extract (mg mL^{-1})	
Microorganism	MIC	MBC	MIC	MBC
B. bronchiseptica	16	32	15	16
Sh. dysenteriae	20	30	18	30
P. mirabilis	-	-	32	32
P. aeruginosa	18	32	-	-

MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration, -: Not measured

DISCUSSION

Medicinal plants could be one approach because most of them are safe with little side effects if any, cost less and affect a wide range of antibiotic resistant microorganisms. The results of this study showed that ethanolic and methanolic extracts from the *Q. brantii* inhibited the growth of various species of Gram-negative bacteria. The *Q. brantii* ethanolic extract (0.2 g mL^{-1}) showed significant effect on *Sh. dysenteriae*. In most countries in the Eastern Mediterranean Region shigellosis is most prevalent in densely populated areas. In addition to endemic shigellosis, some countries have reported in recent years large epidemics of dysentery caused by *Sh. dysenteriae* type 1 (Sd1), which is characterized by a particularly high case fatality rate, extreme debility in survivors and an increasing number of multiple drug-resistant strains (Lichnevski, 1996). The ethanolic extract showed slightly better killing action than the methanolic extract, which means that the ethanolic extract could be used more. Present results are in agreement with observations in previous studies which the alcoholic oak extracts inhibited *P. mirabilis*, *E. coli* and *P. aeruginosa* (Khosravi and Behzadi, 2006). *K. pneumoniae* was resistant to methanolic compound extract. That probably could be due to cell membrane permeability or due to other genetic factors. Tannins could be one of the components responsible for the antibacterial activity since it was reported by other studies that tested different plants (Nimri *et al*., 1999). Based on previous studies on active constituents of oak fruits, tannin is the most abundant compound in the plant whose major effect is anti-diarrhea because of water absorption and protein precipitation (Khosravi and Behzadi, 2006; Voravuthikunchai and Mitchell, 2008). In general, the mechanisms by which microorganisms survive the action of antimicrobial agents are poorly understood and

remain debatable (Okemo *et al*., 2001). Phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds are secondary metabolites of plants that serve as defense mechanisms against predation by many microorganisms, insects and herbivores (Doughari, 2006). In another study, it has been reported that several bioactive flavonoids such as furocoumarins and furanocoumarins (Manderfield *et al*., 1997) also phenolic compounds have been isolated from parsley leaf and are known to exhibit antibacterial activities (Wong and Kitts, 2006; Maleki *et al*., 2008). Furocoumarins can inhibit bacterial growth by reacting with DNA and disrupting DNA replication (Seyyednejad *et al*., 2008), thus explaining the observed growth inhibition of bacterial species in this study. On the other hand, the hydrophobic character of phenolic compounds can potentially impair cellular function and membrane integrity (Raccach, 1984). The capacity of phenolic compounds to chelate transition metals also lowers the reactivity of metal ion by forming an inert metal-ligand complex. Chelation of transition metals, such as iron and copper, reduces bioavailability for bacterial growth (Seyyednejad *et al*., 2008). Despite earlier reports on other properties of the Oak seed hull (Khosravi and Behzadi, 2006), present study was first one on the antimicrobial effect of the Oak fruits in this geographical region. The diameters of inhibition zone around the most active extracts were comparable with the standard antibiotics used as a positive control. The whole Gram-negative bacteria were resistant to Nafcillin.

This study was an *in vitro* investigation without interfering the body 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 due to intervention of these natural factors. Further studies are needed for the clarification of the precise *in vitro* and *in vivo* antimicrobial activities of the plant extracts.

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