

Evaluation of the antibacterial activity of essential oil and aqueous and ethanolic extracts of *Quercus infectoria* leaves on food-borne pathogenic bacteria.

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Abstract

Food-borne pathogens are group of micro-organisms that cause food-borne illness thus, the research for finding effective natural product against this infection is necessary. The purpose of this study was to determine antibacterial activity of essential oil and ethanolic and aqueous extracts *Quercus infectoria* against some important food borne bacteria.

In this study, antibacterial activity of extracts and essential oil was evaluated against bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Yersinia enterocolitica*, *Salmonella typhi* and *Vibrio cholera*) using disc diffusion method. Then broth micro-dilution method was used to determine their minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). The data was expressed as the mean and the standard deviation; and they were statistically analyzed by ANOVA using SPSS software ($P < 0.05$).

Result showed that ethanol extract of *Quercus infectoria* was found to be the most effective against *B. cereus* strains (inhibition zone = 16.85 ± 0.65 mm) than *Yersinia enterocolitica* strains (inhibition zone = 14.15 ± 0.75 mm). Aqueous extract of *Quercus infectoria* was found to be the most effective against *B. cereus* strains (inhibition zone = 7.25 ± 0.45 mm) than *Yersinia enterocolitica* strains (inhibition zone = 14.45 ± 0.21 mm). The MIC values of the essential oil, ethanolic and aqueous extracts against *B. cereus* were 0.064, 0.512, 0.256 $\mu\text{g/ml}$ and against *salmonella typhi* were 0.256, 0.1024, 0.1024 $\mu\text{g/ml}$, respectively.

Essential oil and extracts of *Quercus infectoria* leave showed a good antimicrobial activity against food borne pathogens. Therefore, they can be used in food preservation systems to inhibit the growth of these bacteria and improve food quality and safety.

Keywords: *Quercus infectoria*, Antimicrobial activity, Extract, Essential oil

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. 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(1,2).

In fact during the recent decades, interest in the use of herbal products has increased use of herbal (3,4) Plants play an important role in human health, it is estimated that 25% of modern medicine were derived directly or indirectly from herbs(5). Most plant compound that have been proven effective insecticidal, antifungal, anti-parasitic, anti-bacterial, and anti-viral and anti-oxidants (6,7). Aromatic essential oils are often used as flavoring and prevent the growth of mold and microbial contaminants in food industry (8).

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(9).

Quercus infectoria is a small tree or shrub of about 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(10).

Using of *Quercus infectoria* extracts an essential oil in food preparations, nutraceuticals or cosmetic anti-aging formulations may be promising. Hence, the objective of this study was to assess the antimicrobial activities of *Quercus infectoria* leave in invitro.

Experimental

Preparation of extracts

Quercus infectoria leaves were collected from reclaimed land at Baneh, Orumiyeh province, Iran 2012. 10 g of sample and divided into two equal parts, each of them was cut into small pieces and extracted at room temperature with 100 ml of 80% ethanol (twice after 24 h) for ethanol extract and with 100 ml of water distilled (twice after 24 h) for water extract. The extracts were evaporated under vacuum at 50 °C until dryness.

Extraction of essential oil

The essential oils of the leaves of *Quercus infectoria* were obtained by the steam distillation process using a Clevenger apparatus; 300 g of fruits were placed with sufficient distilled water to cover the material. Extraction continued for 3 consecutive hours after the water had begun to boil.

Disk Diffusion Test

The extracts were tested against *Staphylococcus aureus* ATCC 25913, *Escherichia coli* ATCC 8739, *Bacillus cereus* PTCC 1709, *Salmonella thyphi* PTCC 1709, *Yersinia enterocolitica* PTCC 1477 and *Vibrio cholera* PTCC 1611. The microorganisms were cultured in BHI (Brain Heart Infusion) for 18 hours at 37° C, and resuspended in 0,5 Mac Farland Standard (5 x 10⁸ CFU/mL) and inoculated directly in boards with Mueller-Hinton Agar (Merck). After the inoculation of each microorganism, the diffusion method was used, putting 10µL of essential oil on paper disks (6 mm of diameter) at 37°C/24 hours, after which time the halos of inhibition were measured(11,12).

Micro dilution method

Extracts and essential oil were diluted by using serial micro dilution method with Mueller Hinton Broth culture medium at a final concentration range from 64 to 0.25 %. Both essential oil and extract as assayed for antibacterial activity in triplicate. Before conducting experiments all the conditions were standardized to determine MIC and MBC values in vitro (13).

Statistical analysis

Statistical analysis with SAS software (9.1) was carried out In order to determine significance difference (p< 0.05).

Results and Discussion

The results showed that essential oil of *Quercus infectoria* leave yield is about 0.2 percent. Impact of *Quercus infectoria* leave essential oil and extracts on each of six bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Salmonella thyphi*, *Yersinia enterocolitica* and *Vibrio cholera*) are given in Table1. The highest and lowest zones of growth inhibition at a concentration of 100% are at *Bacillus* and in *Yersinia* respectively (Table2) and also minimum and maximum MIC against bacteria are *Bacillus* and *Staphylococcus* respectively. Also by checking this table result, we conclude that bacteria *Yersinia* and *Salmonella*, together have highest and *Staphylococcus* lowest amount of MBC (Table2).

Many antimicrobial drugs of that some are discovered and some are unknown in the nature. Thus, during the past decade has been an increase in research on plants filed as medicine source.

Essential oils and extracts are potential sources of novel antimicrobial compounds especially against bacterial pathogens. This natural products may also be applied for treatment of infective diseases, so that it is effective as antimicrobial activity (14,15).

In present study was showed that the essential oil and extracts from the leave inhibited the Gram-positive bacteria (*B. cereus* strains) better than Gram-negative (*Yersinia enterocolitica* strains). The MIC values of the essential oil, ethanolic and aqueous extracts against *B. cereus* were 0.064, 0.512, 0.256 µg/ml and against *salmonella typhi* were 0.256, 0.1024, 0.1024µg/ml, respectively.

Generally, plant component are usually more active against Gram positive bacteria than Gram-negative bacteria (16).

The similarity in the antimicrobial activity of the aqueous and ethanolic extracts suggests that these extracts may have high total tannin content. The antimicrobial activity seemed to depend on the contents of tannin in the plant extracts (9).

Gulluce et al reported good susceptible seven strains of different bacteria genera including *Brucella*, *Bacillus*, *Enterobacter*, *Neisseria*, *Pseudomonas* and *Escherichia* to the *Quercus ilex* L. leave extracts (17).

Vermani, et al reported good anti-bacterial activity of *Quercus infectoria* Oliver 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 (18).

Basri et al 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 (19).

Basari 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 antimicrobial activity on the bacterial species (9).

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 (9).

However, in the present study, we have found that plant extract in ethanol provided more consistent antimicrobial activity as compared to those extracted in aqueous. It is probably because various organic compounds can be leached more in this solvent (13,20).

Wasim et al, screened medicinal plants to detect anti-microbial activity and clearly demonstrated that alcohol is a better solvent as compared to water and petroleum ether (20).

Conclusion:

In this study, essential oil and extracts showed significant antibacterial activity. The extracts may be effective in other gram-positive and gram-negative bacteria in food. Therefore it can be good alternative and satisfactory artificial preservatives used in the food industry today.

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Conflict of interest

We declare that we have no conflict of antibacterial activity of essential oil and aqueous and ethanolic extracts of *Quercus infectoria* leaves on food-borne pathogenic bacteria.

Table 2: Determination of MIC and MBC value ($\mu\text{g/ml}$) for aqueous and ethanol extract of Propolis against pathogenic bacterial strains

Test	Y. enterocolitica			S. aureus			V. cholera			B. cereus			E. coli			S. typhi		
	A	E	O	A	E	O	A	E	O	A	E	EO	A	E	O	A	E	EO
	MIC ($\mu\text{g/ml}$)	102 4	51 2	25 6	1 0 2 4		1 2 8	102 4		512 6	2 5 6		256 2 64	204 8	102 4	51 2	204 8	102 4
MBC ($\mu\text{g/ml}$)	204 8	10 24	10 24	5 1 2		2 5 6	102 4	102 4	5 1 2		51 2	128	204 8	102 4	51 2	204 8	102 4	1024

Table 1- comparison of average inhibitory halo diameter (mm) of various bacterial strains for ethanol extract of Propolis

Bacterial strain	Ethanol extract									Aqueous extract								
	10mg/ml			20mg/ml			50mg/ml			10mg/ml			20mg/ml			50mg/ml		
	Min	Max	Average	Min	Max	Average	Min	Max	Average	Min	Max	Average	Min	Max	Average	Min	Max	Average
Staphylococcus aureus	8.7	8.8	8.75±0.37	12.4	12.6	12.5±0.85	16.3	16.7	16.5±0.93	8.3	8.5	8.4±0.25	12.1	12.4	12.25±0.75	15.5	15.8	15.65±0.78
Escherichia coli	7.8	8	7.9±0.30	11.8	12	11.9±0.49	16.1	16.4	16.25±0.57	7.6	7.9	7.75±0.40	11.4	11.5	11.5±0.35	15.3	15.7	15.5±0.23
Vibrio cholera	8	8.1	8.05±0.12	11.4	11.5	11.45±0.55	14.6	14.7	14.65±0.37	7.7	7.9	7.8±0.53	11	11.4	11.2±0.98	16.1	16.5	16.3±0.85
Yersinia enterocolitica	8.5	8.6	8.55±0.25	12	12.3	12.15±0.95	14	14.3	14.15±0.75	8.2	8.5	8.35±0.67	11.4	11.5	11.45±0.45	14.4	14.6	14.45±0.21
Salmonella typhi	8.3	8.5	8.4±0.27	11.5	11.6	11.55±0.38	14.6	14.8	14.7±0.87	8.1	8.3	8.2±0.45	11	11.1	11.05±0.25	15.1	15.4	15.25±0.60
Bacillus cereus	9.6	9.9	9.75±0.45	13	13.2	13.1±0.43	16.7	17	16.85±0.65	9.1	9.3	9.2±0.46	12.4	12.5	12.45±0.33	17.1	17.4	17.25±0.45

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