



The effect of ethanolic and aqueous extracts of *Berberis vulgaris* on multidrug- resistant gram-negative pathogenic bacteria

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ABSTRACT

Background and Aim: In recent years, due to the indiscriminate and irrational use of synthetic drugs, the resistance of pathogenic microorganisms has increased; therefore, new compounds are vitally needed. The purpose of this study was to investigate the effect of ethanolic and aqueous extracts of *Berberis vulgaris* on *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Citrobacter frundi*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in standard conditions.

Material and methods: Ethanolic and aqueous extracts of *B. vulgaris* were prepared by maceration method. After the bacteria were identified, antibiotic resistance was determined by agar disk diffusion method. Antibacterial effect of ethanolic and aqueous extracts of *B. vulgaris* on multidrug resistant bacteria was examined at four concentrations of 50, 100, 400 and 800 mg/ml, and (MIC) and (MBC) of these extracts on macro dilution method was also used. The collected data were analyzed using SPSS Software and Kruskal-Wallis and Mann-Whitney tests.

Results and Conclusion: The results showed that ethanolic and aqueous extracts of *B. vulgaris* had an antibacterial effect on a multi-drug-resistant bacteria (MDR). The MIC and MBC of the extracts were reported 50 and 100 mg/ml, respectively.

Industrial and practical recommendations: After further investigations, extract *B. vulgaris* was recommended to be utilized as an alternative to antibiotics for treatment.

1. Introduction

Bacterial infection is considered as one of the most acute problems by health organizations in different countries. For example, urinary tract infection is one of the most common infections in infants, the elderly and women, which is the most common disease after respiratory diseases (Kiani et al, 2011; Amalaradjou & Venkitanarayanan, 2012). The most important

bacteria that cause the disease can be *Enterobacter Aeruogenesis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Proteus vulgaris* and *Staphylococcus epidermises*. The most common reason for infection is gram-negative bacilli. More than 85% of infections are caused only by *Escherichia coli* (Kumar, 2012).

Treatment is mostly carried out with antibiotics, and if they are frequently used for treatment and prevention of urinary putrefactions, antibiotic resistance will be resulted (Kathleen, 2008). An expanding medical problem increases the strains of pathogenic bacteria that are resistance to antibiotics, which reduces the number of effective antibiotics that are used in treating various infections (Kumar, 2012). In recent years, due to the indiscriminate use of synthetic drugs, the need of new compounds for treatment of resistant pathogenic bacteria has seemed to be essential. Today, researchers are looking for new plant antimicrobial substances to replace with some microorganisms resistance antibiotics. To solve this problem, new medicinal plants and also herbal remedies are used instead of medication, which reduces complications (Kiani et al, 2011; Priti, 2012). *B. vulgaris* is an herb that has a long history of use in traditional medicine. This herb can reduce blood pressure, cause hypoglycemia and have anti-inflammatory effects. Its pharmacological properties such as anti-histamine and anti-cholinergic system have been studied. The *B. vulgaris* is used in the treatment of infectious fever, typhus and dysentery. Some of the quantitative components of *B. vulgaris* can be included Berbamin, oxyacanthine, Jateorrhizine, and berberine which are noted to have therapeutic properties. The alkaloid in root and stem bark of the plant is higher than other parts. There are various combinations in the fruit including Pectin, Polyphenols, Resin, Malic acid and Vitamin C (Kumar, 2012). This study aimed to evaluate the effect of ethanolic and aqueous extracts of *B. vulgaris* on multidrug-resistant gram-negative bacteria which cause urinary tract infection.

2. Material and Methods

2.1. Collect bacterial samples

This study was carried out on 140 different samples of various bacterial strains including *E. coli*, *K. pneumoniae*, *P. vulgaris*, *C. frundii*, *E. aerogenes*, *P. aeruginosa* and *A. baumannii* while all multidrug resistant (MDR) samples were retrieved from Zarrinshahr Vahid medical diagnostic laboratory and also from several hospitals in Isfahan province (Shariati, Saddughee and Gharazy). Moreover the standard of bacteria which was prepared from these

bacteria, was according to Iranian Research Organization for Scienand Technology.

2.2. Preparation of aqueous and alcoholic extracts

Equeous and ethanolic extracts were prepared by maceration method. The aqueous extract was produced by mixing 50 grams of powdered seeds of PP with 100 ml of distilled water while ethanol extract was produced by mixing 50 grams of powdered seeds of PP with 100 ml of 70% ethanol. They were put into a flask for 48 hours on a shaker at degree of 25° C, separately. The extract was separated using a filter-paper pad on a Buchner funnel and dried in sterile glass plates. Therefore, the DMSO series were prepared.

2.3. The effect of ethanolic and aqueous extracts of *B. vulgaris* on bacteria

Extraction with 70% ethanol extract and distilled aqueous extract was done by water soaking method. Various concentrations of aqueous and ethanol extracts of the solvent DMSO (*dimethyl sulfoxide*) were prepared at rates of 50, 100, 400 and 800 mg/ml. Well diffusion method was used to breed different varieties of bacteria such as *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *P. vulgaris*, *E. erugenes*, *A. baumannii* and *C. frundi* on Mueller Hinton Agar for 24 hours. The wells of the culture plate were created with pausteur pipette (6mm). To each of these wells, inoculated concentration of the extract was added and the plates were incubated for 24 hours. Moreover, after bacteria were added to the plates, the standard antibiotics were put into the plates. Dilution method was used for measuring the minimum inhibitory concentration (MIC) in order to avoid the growth of bacteria and microorganisms in the form of the minimum bactericidal concentration (MBC). All the tubes were put on incubator at the degree of 37° C for 24 hours, and then the last tube was considered as the minimum inhibitory concentration of microorganisms because no growth was observed on it. Additionally, Gentamycin disc was used as the standard control antibiotic (Kognou et al., 2011).

From among all the tubes that were without turbidity, a calibrated loop was cultured on a blood agar medium. Last dilution of the extract which was able to kill 99.9% of the initial viable bacteria was considered as the minimum concentration bactericidal microorganism (Kiani et al., 2011). Each

of the experiments was repeated three times and the collected data were analyzed using Kruskal-Wallis

and Mann-Whitney tests through SPSS Software.

3. Results and Discussion

Agar plate method results of the effect of different concentrations of aqueous extract of *B. vulgaris*

Table 1. Mean and standard deviation of the zone of growth inhibition of different concentrations of aqueous extract of *B. vulgaris* on multidrug-resistant gram-negative bacteria causing urinary tract infection

concentrations (mg/ml)		800	400	100	+Control
bacteria	Number of	Mean	Mean	Mean	Mean
<i>Acinetobacter baumannii</i>	20	15.38±1.794	11.48±2.919	--	13.71±4.50
<i>Pseudomonas aeruginosa</i>	20	27.47±4.354	22.81±2.359	11.83±2.841	8.31±8.49
<i>Citrobacter frundi</i>	20	21.21±2.443	15.29±.486	10.58±3.028	9.95±6.59
<i>Proteus vulgaris</i>	20	17.23±2.320	12.57±2.357	--	15.64±2.69
<i>Escherichia coli</i>	20	25.97±4.893	19.87±4.499	9.40±3.689	13.71±6.10
<i>Enterobacter aerugenes</i>	20	21.83±2.278	15.60±1.741	8.17±2.768	13.73±4.94
<i>Klebsiella pneumoniae</i>	20	19.12±3.810	12.40±3.657	--	10.00±6.72

Based on the results presented in Table 1 and Figure 1, the aqueous extract of *Berberis vulgaris* was only effective on the concentrations of 50, 100, 400 and 800 mg/ml on *A. baumannii*, *P.vulgaris*, *K.pneumoniae*, *C. freundi*, *E. coli*, *P. aeruginosa* and *E. aerugenes*. In addition, it was effective in the

concentration of 100 mg/ml for *A. baumannii*. The highest average diameter of inhibition zone in concentration of 800 mg/ml for the *P.bacteria* is 27.47 mm while the lowest is for *A. baumannii* which is 15.38.

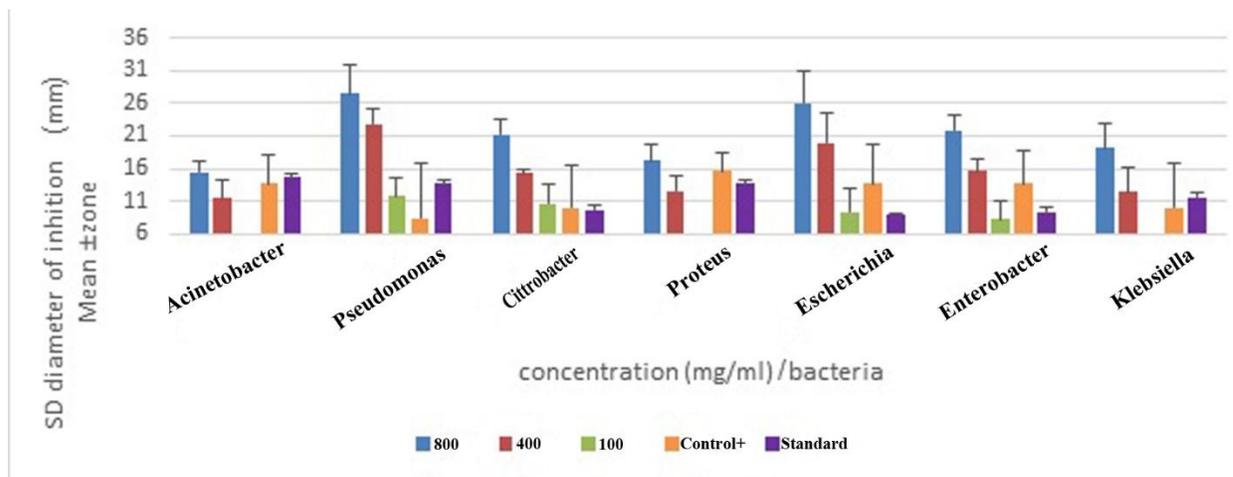


Fig 1. Mean ± SD diameter of inhibition zones of different concentrations of aqueous extract of *Berberis vulgaris* on multidrug-resistant gram-negative bacteria causing urinary tract infection

Agar plate method results of the effect of different concentrations of ethanol extract of *B. vulgaris*

Table 2. Mean and standard deviation of the zone of growth inhibition of different concentrations of ethanolic extract of *B. vulgaris* on multidrug-resistant gram-negative bacteria which causes urinary tract infection

concentrations (mg/ml) Bacteria	Number of	800	400	100	+Control
		Mean	Mean	Mean	Mean
<i>Acinetobacterbaumannii</i>	20	15.38±1.794	11.48±2.919	-	13.71±4.50
<i>Pseudomonasaeruginosa</i>	20	27.47±4.354	22.81±2.359	11.83±2.841	8.31±8.49
<i>Citrobacterfrundi</i>	20	21.21±2.443	15.29±0.486	10.58±3.028	9.95±6.59
<i>Proteus vulgaris</i>	20	17.23±2.320	12.57±2.357	-	15.64±2.69
<i>Escherichia coli</i>	20	25.97±4.0839	19.87±4.499	9.40±3.689	13.71±6.10
<i>Enterobacteraerugeness</i>	20	21.83±2.278	15.60±1.741	8.17±2.768	13.73±4.94
<i>Klebsiellapneumoniae</i>	20	19.12±3.810	12.40±3.657	-	10.00±6.72

Based on the results shown in Figure 2 and Table 2, ethanol extract of *B. vulgaris* was only effective on *K.pneumoniae* at concentrations of 400 and 800 mg/ml and *A. baumannii* in Bacteria, *C. frundi*, *P. vulgaris*, *E. coli*, *Pseudomonas aeruginosa* and *E. aerugeness* bacteria. Moreover, the concentration of 100 mg/ml was effective, too.

The diameter of inhibition zone for each of the concentrations of 100, 400 and 800 mg/ml of the bacteria were compared using Kruskal-Wallis test. Based on the results obtained in the concentration of 400 mg/ml, there was no significant difference

between the diameter of inhibition zones of the seven bacteria (0.087 = p) (or (0.05<p)).

However, at concentrations of 100 and 800 mg/ml, a significant difference between the diameter of inhibition zones was observed in the seven bacteria (0.05> p).

Test results showed that in the concentration of 400 mg/ml, there was no significant difference in the diameter of inhibition zone growth between the *A. baumannii* and *E. aerugeness*, but the diameter zone of *A. baumannii* was more than *E.aerugeness*.

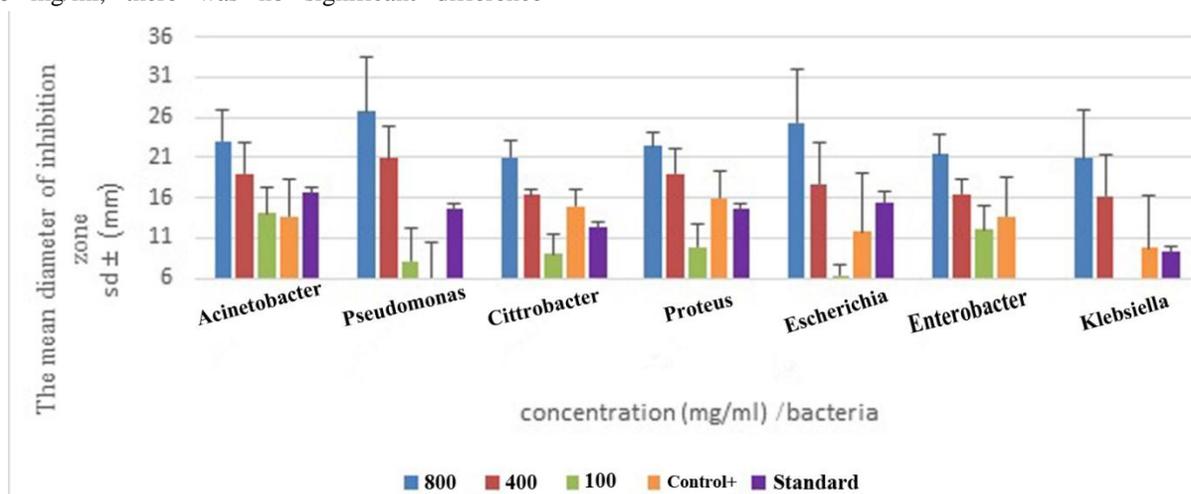


Fig 2. The mean diameter of inhibition zone of the ethanolic extract of *B. vulgaris* on multidrug-resistant gram-negative bacteria causing urinary tract infections.

E. coli is one of the most bacterial species isolated from human infections and the most common cause of urinary tract infection and sepsis caused by Gram-negative bacilli and other infections including wound infections and pneumonia. The bacteria that produce beta-lactamase due to the acquisition of broad-spectrum plasmid that encodes a number of broad-spectrum antibiotics such as cephalosporins were

resistant. Therefore, treatment of infections according to laboratory diagnostics is easy to fail. Many researchers have stated that urinary tract infections are *Escherichia coli* etiologies, and 85% of this type of infection accounts. In the past, ampicillin and amoxicillin were among the prior antibiotics used to treat urinary tract infections, but with the rapid expansion of drug resistance in strains of *E.coli*, these

antibiotics lost their effectiveness in the treatment of urinary tract infections (Motamedi et al, 2010).

In a study, it was figured out that *E. coli* had the highest frequency allocated to the antibiotics *ampicillin* and *ofloxacin* 100% resistant to *amoxicillin*. UTI etiological factors are often part of the Enterobacteriaceae family of bacteria in the urinary tract infections, and *K. pneumoniae* causes 17-16% of *E. coli* as the second most common cause of urinary tract infection. *K. pneumoniae* has been one of the most important bacteria in recent decades due to incorrect use of the procedure and the emergence and spread of antibiotic resistant strains with multiple drug resistance (Kumar, 2012). In the present study, *K. pneumoniae* had the highest resistance to antibiotics *ampicillin*, *amoxicillin*, *tetracycline* and *ciprofloxacin*, *ofloxacin*.

Pseudomonas aeruginosa as an important factors in nosocomial infections, particularly in patients treated wounds, surgical infections and burns the bacteria may be *septicemia*, *pneumonia*, *meningitis* and other fatal diseases may follow. Increasing antibiotic resistance in *P. aeruginosa* created many problems for patients who had faced with a lot of problems in treatment and increased morbidity and mortality. Specific resistance of bacteria to antibiotics gained increased

Pseudomonas aeruginosa bacteria resistant to most antibiotics led to the spread of antibiotic use and hospital infections caused by these bacteria help. The present study showed that the bacteria is resistant to antibiotics like *clindamycin*, *ampicillin*, *amoxicillin* and *ceftriaxone* (100%).

Evidence collected showed *E. aerogenes* and *P. vulgaris* tertiary clinical significance of bacteria that cause urinary tract infection and the highest resistance to the antibiotic amoxicillin (100%). The results showed that the bacterium *C. frindii* had a lower clinical importance in the resistance of the bacteria to some antibiotics in treating urinary tract infections caused by them. In the present study, the two antibiotics *ampicillin* and *amoxicillin* resistance equivalent (100%) showed an increasing antimicrobial resistance of the bacteria to antibiotics and prevalence of infectious diseases, which requires the usage of proper antibiotic, the ability of some plants to produce antimicrobial substances, caused the researchers to consider the extracts as a safe replacement to antibiotics (Motamedi et al, 2010).

This study aimed to evaluate the antimicrobial properties of aqueous and ethanol extract of *B. vulgaris*. The effects of ethanolic and aqueous extract of *B. vulgaris* at different concentrations, with considering the inhibition zone of tested gram-negative bacteria were relatively acceptable at different concentrations.

In 2012, Kumar reported that the root methanol extract of *B. vulgaris* has a good antimicrobial activity against *E. coli*, *S. typhimurium*, *K.pneumoniae* and *P. aeruginosa*.

Paradeep et al. studied the antibacterial activity of the stems and roots extract of *B. vulgaris* on *E. Coli* and *S. aureus* and compared its effect to antibiotic *ampicillin* tri-hydrate, according to Berberin alkaloid found in *berberis*, they concluded that the extracts have a strong antibacterial activity.

Pasryja et al. in 2011, studied the *B. aristata* antibacterial activity of bacteria. In a study, this type of *berberis* was collected from different regions of India. After extraction, its effect on the *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *B. subtilis*, *E coli* and *Aspergillus niger* bacteria was studied. According to the report of that study, the Berberin alkaloid was present in all samples and this composition had a great antimicrobial effect on *B. aristata*.

In our study, the MIC and MBC of the ethanolic and aqueous extract of *B. vulgaris* was respectively 50 and 100 mg/ml for most bacteria.

Gareeb et al.(2013) investigated the anti-bacterial, anti-fungal and anti-viral effect of ethanol extract of *B. vulgaris*. They reported that the ethanol is an effective extract on Gram-positive and Gram-negative range in 2.5-20 mg/ml, on the *Aspergillus flavus* fungi based on inhibition of aflatoxin B1 and B2 at concentrations of 0.01-0.1, and about the hepatitis type c, concentration in the only inhibitory dose 100µg/ml of ethanol extract of *barberry*. Kiani et al (2011) examined the effect of ethanol extracts of seven species of medicinal plants on bacteria isolated from patients with urinary tract infection in Gorgan. They reported that extracts of fruits and leaves of *B.vulgaris* showed the best effect on gram-positive bacteria. In our study, ethanolic and aqueous extract of *B. vulgaris* had a significant effect on multidrug-resistant gram-negative bacteria which was similar to other studies, except for Kiani's investigation. This difference may be due to different extractions from those of the present study. Moreover, in the present

study soaking method was used for extracting while Kiani used decanter for that purpose. In our research, fruit extract was separated while Kiani *et al* extracted it from the fruit and leaves of *barberry*. Moreover, the variety of *B. vulgaris* is important.

4. Conclusion

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In general, the present study showed that the antibacterial effect of ethanolic and aqueous extract of *B. vulgaris* is effective on multidrug-resistant gram-negative bacteria including *E. coli*, *K. pneumoniae*, *P. vulgaris*, *C. frundi*, *E. aerogenes*, *P. aeruginosa* and *A. baumannii*.

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