

In vitro antibacterial activity of *Bunium persicum* and *Rheum ribes* on *Acinetobacter baumannii*

Research Article

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Abstract

Introduction: Using antibiotics to control pathogenic bacteria is associated with certain side effects in addition to emergence of drug resistance. Nowadays, researchers are considering using plants as suitable alternatives to antibiotics. The aim of study was to compare in vitro antimicrobial activities of aqueous and hydroalcoholic *Bunium persicum* and *Rheum ribes* L. extracts on *Acinetobacter baumannii*. **Materials and methods:** Different concentrations of *R. ribes* and *B. persicum* were prepared to determine microbial sensitivity of *A. baumannii* using disk diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC). **Results:** The highest MIC (256 µg/ml) against *A. baumannii* was derived for aqueous *R. ribes* and *B. persicum* extracts and the lowest MIC (128 µg/ml) for their hydroalcoholic extracts. The highest MBC (512 µg/ml) was derived for aqueous *R. ribes* extract and the lowest MBC (256 µg/ml) for aqueous and hydroalcoholic *R. ribes* and *B. persicum* and hydroalcoholic *R. ribes* extracts. The extracts exhibited great inhibitory effects against the studied bacteria in disk diffusion; and with increasing the extracts' concentrations, the inhibitory effect was exhibited more markedly as increased diameter of inhibition zone. **Conclusion:** Hydroalcoholic *R. ribes* and *B. persicum* extracts can be used as nature-based compounds to control *A. baumannii*.

Keywords: Plant extract, antimicrobial activity, *Acinetobacter baumannii*, *Bunium persicum*, *Rheum ribes*.

Introduction:

Acinetobacter baumannii is a gram-negative, non-motile, and non-fermented coccus that is isolated mainly in hospital settings and ICUs. It is argued that this opportunistic pathogen is associated with nosocomial infections including urinary tract infections (UTIs), surgical site infections, meningitis, and occasionally infections of skin and central nervous system (1,2). This bacterium can grow in a wide range of pH and environmental temperature and uses different types of substances to grow (3). *A. baumannii* is widely known mainly because it can survive in both moist and dry environments, and on unsterile medical devices such as ventilators, catheters, and respiratory equipment (4). Due to wide and frequent therapeutic treatments, this pathogen has acquired resistance to drugs, even strong ones, across the world. Although pharmaceutical and pharmacological industries have produced some new antibiotics during the last three decades,

microorganisms' resistance to these antibiotics has increased. Overall, it is obvious that bacteria can genetically be converted and resist therapeutic agents. In addition to increasing healthcare costs, this multidrug resistance has increased mortality rate and its spread to the environments outside hospital settings (5). This issue should be seriously and even worriedly taken into consideration, especially for immunosuppressive patients who are faced with certain bacteria in hospitals that acquire double and even greater resistance, and therefore development of new infections can lead to higher rates of mortality (6). The problem with such microbial resistance is uncertainty about antimicrobial drugs in the future. Therefore, certain measures should be designed to decrease this problem including controlling use of antibiotics, developing research to better understand the genetic mechanism of this resistance, and conducting research to develop new drugs (7). Currently, more potent therapeutic agents should be sought out and used to fight antibiotic resistant bacteria particularly extensive drug resistant (XDR) ones including *A. baumannii*. Using medicinal plants is a strategy to achieve this purpose. Certain medicinal plants have been used to develop such therapeutic methods, due to bioactive compounds present in them, to investigate their potential use. For

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thousands of years, plant extracts have been used for various purposes. Particularly, many applications such as preservatives, pharmaceutical items, alternative medicine, and nature-based therapies have been based on the antimicrobial activities of plant essences and extracts. Therefore, it would be a promising strategy to discover medicinal plants with antimicrobial effects that are economical due to causing comparatively fewer side effects and exerting less toxicity on tissues (8).

Rhubarb, botanically referred to as *Rheum Ribes L.*, is from family Polygonaceae. Different species of this plant occur in different regions of the world including China, India, Turkey, and Iran. *R. ribes* is a type of resistant perennial plant that is cultivated in some countries due to its edible red part (9). *R. ribes* occurs in different regions of Iran especially Mashhad and Nishapur. In traditional medicine books, several properties have been mentioned for *R. ribes* including appetizing, blood-purifying, bile reducing, gastrointestinal tract especially liver-enhancing, bile and phlegm-excreting, hepatoprotective, and antidotic for certain toxins (10). This plant has been frequently studied and reported to have various properties including antiplatelet accumulation, hypolipidemic, kidney failure and disease-treating, pancreatic tissue-improving in diabetic people, and fat accumulation-reducing in the liver (11).

Black cumin (*Bunium persicum*) is an aromatic perennial plant from family Umbelliferae that occurs from Central Asia to northern India. Its seeds are widely used as spice. In traditional medicine, it is used as anti-flatulence agent as well as in treating indigestion (12). *B. persicum* has a great potential for pharmaceutical applications due to antispasmodic, cancer-preventive, cell proliferation-controlling, acid secretion-reducing, mucin secretion-increasing, prostaglandin 2-increasing, and leukotriene-reducing (13). *B. persicum* widely occurs in the regions of Iran with dry climate such as Kerman, Fars, Isfahan, and Yazd provinces (14).

With regards to the significance of *A. baumannii* in spreading diseases and importance of using phytotherapy as well as because no study has yet been conducted to investigate in vitro antibacterial effects of *R. ribes* and *B. persicum*, this study was conducted to investigate the antibacterial effects of hydroalcoholic and aqueous *R. ribes* and *B. persicum* aerial parts extracts on *A. baumannii*.

Materials and methods

Extraction

To prepare hydroalcoholic extract, 500 g of the studied plants were pulverized with a mill and extracted by maceration. To do this, 500 g of each plant was kept in combination with alcohol 70% in 1-liter volume for four days. After being filtered with Whatman filter paper, the solution was concentrated in rotary at 40 °C. After distillation, the solution was incubated at 37°C and the extract obtained. Then, a stock solution was prepared from the extract using dimethyl sulfoxide.

Preparation of microbial suspension

Twenty four hours before the study, a fresh (24-hour) culture medium was prepared by means of already prepared culture media such that before onset of inoculation, an amount of the colonies on the surface of Mueller-Hinton broth was transferred into a physiological serum-containing tube with a sterile swab and then microbial suspension opacity prepared and compared with 0.5 McFarland standard opacity.

Bacteria and antibiotics

Standard *A. Baumannii* strain (ATCC747), purchased from Iranian Research Organization for Science and Technology as lyophilized, was used in this study. The antibiotics used in this study were amikacin, imipenem, ceftazidime, gentamicin, and ciprofloxacin (Padtan Teb Co., Iran).

Tests of determining drug sensitivity (antibiogram)

To conduct antibiogram, disk diffusion was used. For this purpose, paper sterile blank disks (Padtan Teb Co., Iran) were placed in prepared concentrations of the extract for 24 hours so that the extract was completely absorbed by the disks. Then, the disks containing the extracts in various concentrations were incubated at 37 °C for one hour to dry and then cultured on the plates containing bacteria alongside the standard disks containing amikacin, imipenem, ceftazidime, gentamicin, and ciprofloxacin at appropriate distances and incubated at 37 °C for one hour to examine the extract for antibacterial activity. Finally, inhibition zone diameters were measured by ruler. To achieve this purpose, the ruler's zero edge was placed next to the diameter of the inhibition zone to measure the distance to where the inhibition zone end was placed on the ruler. The inhibition zone diameters were reported in mm and the results were compared with CLSI2013.

Determining minimum inhibitory concentration (MIC)

MICs were determined according to the CLSI protocol (2013). First, a stock solution was prepared from the extract and then the extract was diluted in ELISA wells containing TSB culture medium in serial manner (1/2 dilution). After preparing bacterial suspension according to 0.5 McFarland Standard, 100 µl of the bacterial suspension was added to each well and incubation was performed at 37 °C for one hour. After bacterial growth, the MICs were determined.

Determining minimum bactericidal concentration (MBC)

After determining the MICs, 20 µl of the solutions of the tubes whereby the bacteria did not grow was removed and cultured on blood agar medium. After 24-hour incubation at 37 °C, the least concentration of the extract in which the bacteria was unable to grow was considered MBC.

Statistical analysis

The diameters of all inhibition zones for *R. ribes*, *B. persicum*, and positive control were measured and analyzed by comparing mean values. All tests were conducted in triplicate.

Results

In this study, the antimicrobial activities of aqueous and hydroalcoholic *R. ribes* and *B. persicum* extracts were measured on *A. baumannii*; and the inhibitory effects of these extracts were measured with reference to presence or absence of inhibition zone, and the MICs and MBCs were measured.

The results on hydroalcoholic *R. ribes* and *B. persicum* extracts indicated that the MIC and MBC of both extracts were 128 and 256 µg/ml, respectively, for *A. baumannii*. In addition, the results on aqueous *R. ribes* and *B. persicum* extracts showed that the MIC of both extracts was 256 µg/ml, and the MBC of *R. ribes* extract was higher (512 µg/ml) (Table 1).

Table 1. Effect of hydroalcoholic and aqueous extracts of the plants investigated on *Acinetobacter baumannii*

Plant	Hydroalcoholic extract		Aqueous extract	
	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)
<i>Bunium persicum</i>	128	256	256	256
<i>Rheum ribes</i>	128	256	256	512

The highest MIC (256 µg/ml) against *A. baumannii* was derived for aqueous *B. persicum* and *R. ribes* extracts and the lowest MIC (128 µg/ml) for their hydroalcoholic extracts. The highest MBC (512 µg/ml) was derived for aqueous *R. ribes* extract and the lowest MBC (256 µg/ml) for aqueous and hydroalcoholic *B. persicum* and hydroalcoholic *R. ribes* extracts. The MIC and MBC of hydroalcoholic *B. persicum* extract were lower than those of aqueous *R. ribes* extract.

In disk diffusion, the inhibition zone diameters in different groups (Tables 2 and 3) indicated that the greatest effect was exerted by hydroalcoholic *B. persicum* extract (28 mm) and the least effect by aqueous *R. ribes* extract in 512 µg/ml (7 mm). Inhibition zone diameter was directly correlated with the extracts' antibacterial activities. No inhibition zone was seen surrounding the blank disk that was considered to represent negative control.

Table 2. Zone diameters of growth inhibition (in mm) caused by aqueous *Bunium persicum* and *Rheum ribes* extract at different concentrations with disk diffusion method

Different concentrations Extract	8 (µg/ml)	16 (µg/ml)	32 (µg/ml)	64 (µg/ml)	128 (µg/ml)	256 (µg/ml)	512 (µg/ml)
<i>Bunium persicum</i>	-	-	-	-	-	23 mm	27 mm
<i>Rheum ribes</i>	-	-	-	-	2 mm	4 mm	7 mm

*-Demonstrate bacterium resistance

Gentamicin:15mm, Imipenem:16mm, Amikacin:17mm, Ceftazidime:18mm, Ciprofloxacin:21mm

Table 3. Zone diameters of growth inhibition (in mm) caused by hydroalcoholic *Bunium persicum* and *Rheum ribes* extract at different concentrations with disk diffusion method

Different concentrations Extract	8 (µg/ml)	16 (µg/ml)	32 (µg/ml)	64 (µg/ml)	128 (µg/ml)	256 (µg/ml)	512 (µg/ml)
<i>Bunium persicum</i>	-	-	-	-	15 mm	20 mm	28 mm
<i>Rheum ribes</i>	-	-	2 mm	4 mm	8 mm	10 mm	12 mm

*-Demonstrate bacterium resistance

Gentamicin:15mm, Imipenem:16mm, Amikacin:17mm, Ceftazidime:18mm, Ciprofloxacin:21mm

Discussion

Experimental evidence and using plants as drugs offer basic information on usefulness of plant extracts in special medical conditions. Since the past, plant extracts such as those of tea tree and clove tree have been used for topical treatment due to antimicrobial properties (15,16). It is essential to examine the antimicrobial properties of such plants that have already been used in traditional medicine (17). In addition, using the compounds that are welcome by consumers, nature-based, and safe further highlights the need for supplementing information on these plant-based extracts and compounds. Studies have confirmed the activities of the essences and extracts derived from various plants such as rosemary, peppermint, basil, tea tree, celery seeds, and fennel (8,18).

The current study demonstrated that hydroalcoholic *R. ribes* and aqueous and hydroalcoholic *B. persicum* extracts exhibited adequate antibacterial activity against *A. baumannii*. The greatest MIC against *A. baumannii* was derived for aqueous *B. persicum* and *R. ribes* extracts and the lowest MIC for their hydroalcoholic extracts. The highest MBC was derived for aqueous *R. ribes* extract and the lowest MBC for aqueous and hydroalcoholic *B. persicum* and hydroalcoholic *R. ribes* extracts.

It has been reported that generally, phenolic compounds are active compounds of plant extracts against *A. baumannii*, a gram-negative bacterium (19). Many compounds isolated from plants whose activities have been demonstrated are secondary compounds that have been isolated using solvents such as methanol, ethanol, water, and acetone as well as by different techniques. A study on antibacterial activity of alcoholic *R. ribes* extract against microorganisms from genera *Escherichia coli*, *Pseudomonas*, *Shigella*, *Bacillus*, *Staphylococcus*, and *Micrococcus*, demonstrated that *R. ribes* exhibited antimicrobial activity (20). Antimicrobial agents are certain polyphenols such as simple phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins, and coumarins. Besides that, terpenoids, essential oils, alkaloids, lactones, polypeptides, etc. have been reported. The action mechanisms of these compounds depends on their types. These mechanisms include inhibiting enzymatic activity, plant extract active compounds' combining with extracellular proteins, and solving microbe's or compound's cell with bacterial cell wall (21). Certain extracts can disrupt microorganism DNA, which may occur as development of ion channels in the microbial membrane or competing with the microbial proteins for binding to the host's polysaccharide receptors (22).

A study on cinnamon's activity against drug resistant *A. baumannii* demonstrated that cinnamon's aldehydes prevented the decarboxylating activities of amino acids in bacteria but could not degenerate extracellular membrane or evacuate intracellular ATP (21). Hydroxyl groups of phenolic compounds can

degenerate bacterial cell wall membrane. More clearly, these groups can decrease the amount of intracellular ATP and membrane potential (23). In addition, the secretion of certain substances such as ATP, amino acids, ions, and nucleic acids can ultimately lead to bacterial death. Turmeric has been reported to exert antibacterial activity against *Acinetobacter* for certain reasons such as disrupting folic acid metabolism and cell wall gap (24).

R. ribes has already been demonstrated to exert significant antimicrobial effects on bacterial genera such as *Pseudomonas*, *Klebsiella*, *E. coli*, etc. (9). Cumin exhibited potent inhibitory effect against *Listeria monocytogenes*. It has been argued that the terpenes in cumin essence caused inflammation of cytoplasmic membrane and disruption of microorganism membrane (12).

Karaman et al. studied antimicrobial activity of alcoholic *Juniperus oxycedrus* leaf extract on a wide spectrum of bacteria including gram-negative, gram-positive, and fungal bacteria. Their results demonstrated that aqueous *Juniperus oxycedrus* extract had no antimicrobial activity against bacterial and fungal microorganisms. The highest MIC and MBC of alcoholic extract of this plant ranged 7-17 mm for sensitive microorganisms (25). In addition, evidence has indicated that ethanol is a better solvent to isolate the antimicrobial compounds from medicinal plants compared to other solvents such as water and hexane (26). Differences in the sensitivity rates of microorganisms against antimicrobes in plant extracts can be explained by different cell wall compounds or the specific genetics of the species in plasmid that can easily vary among different bacterial species (25). It is relatively difficult to compare the current study's findings with other's, which can be due to different compounds in different plant-based essences and extracts possibly because of native climatic and ecological conditions of the plants. In addition, it is likely that some compounds with conventional similar names are isolated from different plants, and the methods of assessing antimicrobial activity and selected tests differ among findings (21).

Briefly, this study confirmed that these extracts, with antibacterial properties and if further studied, can be used as antibacterial agents for treating infectious diseases. As well, with regards to the effects of these extracts on life and proliferation of *A. baumannii*, they can be used as drugs to prevent the wounds due to this bacterium.

Conclusion

This study was first to show moderate to high antibacterial effects of hydroalcoholic and aqueous *R. ribes* and *B. persicum* extracts that varied with changing the extracts' concentrations. Aqueous extracts exerted the greatest effect and can therefore be used as a potent

antimicrobial agent, drug supplement, and alternative treatment. Moreover, it is essential to conduct further experimental studies and clinical trials to investigate inhibitory effects of these extracts on *A. baumannii*.

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Conflict of interest

There are no conflicts of interest in this study.

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