

Activity of Some Iranian Plant Extracts against Multi-Drug Resistant Human Pathogens Isolated from Urinary Tract Infections

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Article information	Abstract
<p>Article history: Received: 4 Aug 2013 Accepted: 1 Sep 2013 Available online: 26 Oct 2013 ZJRMS 2014 Oct; 16(10): 50-54</p> <p>Keywords: Antibacterial activity Extract plant Human pathogens</p> <p>*Corresponding author at: Department of Agronomy and Plant Breeding, Agriculture Research Center, Zabol University, Zabol, Iran. E-mail: s.saeidi12@yahoo.com</p>	<p>Background: Plants used for traditional medicine contain a wide range of substances which can be used to treat various infectious diseases. Hence, antibacterial activities of ethanol extracts of 6 plant species were studied against multi-drug resistant clinical isolates.</p> <p>Materials and Methods: A cross-sectional study was performed. Plant extract from leaf of <i>Marrubium vulgari</i>, <i>Saturja montana</i>, <i>Myrtus comminus</i> L., <i>Amaranthus retraxiflexus</i>, seed of <i>Cumminum cyminum</i> L. and <i>Peganum harmal</i> specie was performed using rotary. Sampling was carry out from urine culture of hospitalized patients (Boo-Ali hospital, Zahedan, south-eastern of Iran) suffered from urinary tract infections during the years 2010 and 2011. Minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) of plant extracts of plants on bacteria was determined using micro dilution broth method at 6 different concentrations.</p> <p>Results: The results show <i>P. harmal</i> and <i>M. comminus</i> L. were a potent antimicrobial activity against Gram-positive (<i>Staphylococcus aureus</i>) and Gram-negative (<i>Escherichia coli</i> and <i>Enterobacter cloacae</i>) bacteria respectively. Moreover, all 6 plants extracts showed relatively same antibacterial activity against Gram-positive (<i>Staphylococcus saprophyticus</i> and <i>S. aureus</i>) and <i>S. Montana</i> extracts showed relatively same antibacterial activity against all Gram-negative bacteria and <i>Morganella morgani</i> was the more resistant bacteria for all plants extracts.</p> <p>Conclusion: This investigation showed that the mixes of <i>P. harmal</i> and <i>M. comminus</i> L. extracts have a potent antimicrobial activity against some Gram-positive pathogenic and Gram-negative bacteria. The present studies confirm the use of this extracts as antibacterial agent. Further research is required to evaluate the practical values of therapeutic applications.</p> <p>Copyright © 2014 Zahedan University of Medical Sciences. All rights reserved.</p>

Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections affecting humans throughout their life span [1]. The organism *Saturja montanas* Gram-positive most commonly responsible for UTIs are *Staphylococcus saprophyticus*, *Enterococcus faecalis*, the Gram-negative organisms: *Escherichia coli*, *Klebsiella pneumonia*, *Proteus* and *Providencia* species, *Pseudomonas aeruginosa*, *Enterobacter* and *Serratia* species, *Morganella morganii* [2] and serious causes include *Staphylococcus aureus* and *Candida* species [3]. The development and the increase of antimicrobial resistance among microbial pathogen causing nosocomial and community-acquired infections in known to be associated with the level of antibiotic use. Nosocomial infections caused by *P. aeruginosa* most frequently involve the respiratory tract, the urinary tract and wounds an increasing proportion of *P. aeruginosa* infection have become resistant to antibiotics such as carbapenem [4]. *S. aureus* infections are widespread in the USA, and commonly manifest as minor skin and soft tissue infections such as boils, abscesses, furuncles, folliculitis and cellulitis [5]. An increasing proportion of

S. aureus infections have become resistant to antibiotics such as methicillin, penicillin, and cephalixin; these infections, known as methicillin-resistant *Staphylococcus aureus* (MRSA), are often referred to as a 'superbug' by the media [5]. *Morganella morgani* belongs to the tribe proteeae of family Enterobacteriaceae. Since then, there have been various reports of this organism causing urinary tract infections, skin and soft tissue infections, meningitis and bacteremia often with fatal consequences [6]. Citrobacter Gram-negative coliform bacteria in the Enterobacteriaceae family. *Citrobacter freundii* has recently been reported to express resistance to broad-spectrum antibiotic including piperacillin, piperacillin-tazobactam, vancomycin and cephalosporins [7]. *E. coli* is one of the most common bacteria capable of causing infection in humans, particularly urinary tract infections [8].

The strains of *E. coli* isolated from hospitalized patients were more resistance to amoxicillin. Amoxicillin-clavulanate, trimethoprim, ciprofloxacin, cephalixin and gentamicin compared to those from outpatients [9]. Traditional medicine is in practice for many centuries by

a substantial proportion of the population of many centuries. It is recognized that in some developing countries, plants are the main medicinal source to treat various infectious diseases [10]. In the last few years a number of studies have been conducted to verify the effectiveness of plant extracts against bacterial infections. The antimicrobial properties of various plants have been investigated by number of researchers. Antimicrobial activity of some medical plants against *Bacillus subtilis*, *E. coli*, *S. aureus*, *P. aeruginosa*, *Proteus vulgaris* and *Klebsiella pneumonia* were screened and showed that, among these bacteria, *E. coli*, *P. vulgaris* and *S. aureus* were highly inhibited. The aim study evaluation of antimicrobial activity of the leaf and seed extract of plants against selected pathogens isolation from urinary tract infections.

Materials and Methods

In this cross-sectional study, the leaf of *M. vulgari*, *S. montana*, *M. comminus* L., *A. retriflexus* and seed of *C. cyminum* L. and *P. harmal* were collection in the region of Iran (Zahedan and Kerman, south-eastern of Iran) and plant in Kerman Azad University herbarium received approval and dried at room temperature (Table 1). Samples were crashed and transferred into glass container and preserved until extraction procedure was performed in the laboratory.

Plants were properly dried and pulverized into a coarse powder as reported by Hanafy and Hatem [11]. Each of 20 g grinded powders was soaked in 60 mL ethanol 95%, separately for 1 day. After 1 day of dissolving process, materials were filtered (Whatman no. 1 filter paper). Then the filtrates were evaporated using rotary evaporator. At last, 0.97 g of dried extracts were obtained and then stored at 4°C in air tight screw-cap tube.

Clinical isolates of the following bacteria: *S. aureus*, *S. saprophyticus*, *S. epidermidis* and negative-bacterial *E. Coli*, *E. cloacae*, *P. aeruginosa*, *M. morgani* and *C. freundii* were isolated from urine culture of hospitalized patients (Boo-Ali hospital, Zahedan, south-eastern of Iran) suffered from urinary tract infections during the years 2010 and 2014 (Table 2).

All bacteria were cultured on nutrient agar plates and were incubated for 24 h at 37°C. Few colonies from these cultures were inoculated into Mueller-Hinton Broth and incubated at 37°C for 24 h before use. Nutrient agar (Merck) was used to maintain the clinical isolates of the bacteria.

The broth micro dilution method was used to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) [12]. All tests were performed in Mueller Hinton Broth supplemented with Tween 80 at a final concentration of 0.5% (v/v). Briefly, serial doubling dilutions of the extract were prepared in a 96-well microtiter plate ranged from 0.3 mg/mL to 10.00 mg/mL. To each well, 10 µL of indicator solution (prepared by dissolving a 10 mg extract in 2 mL of DMSO) and 10 µL of Mueller Hinton Broth were added.

Finally, 10 µL of bacterial suspension (10^6 CFU/mL) was added to each well to achieve a concentration of 10^4 CFU/mL. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plated were prepared in triplicates, and then they were placed in an incubator at 37°C for 18-24 h. The color change was then assessed visually. The lowest concentration at which the color change occurred was taken as the MIC value. The average of 3 values was calculated providing the MIC and MBC values for the tested extract. The MIC is defined as the lowest concentration of the extract at which the microorganisms does not demonstrate the visible growth. The microorganisms growth was indicated by turbidity. The MBC was defined as the lowest concentration of the extract at which the incubated microorganisms was completely killed.

Antibacterial activity of extract of plants was tested using the agar well diffusion method [13]. The test inoculum (0.5 McFarland's turbidity) was spread onto Muller-Hinton agar by using a sterile cotton swab. Then the wells were made by a sterile well puncture. Twenty microliters of extracts were added to each well and incubated at 37°C for 24 h. The presence of zones of inhibition was regarded as the presence of antimicrobial action. The diameter of zone of inhibition was measured in millimeter. From the inhibition zones seen, antimicrobial activity was expressed in terms of average diameter of the zones inhibition measured.

The results were expressed as mean and or ranked in order of importance as percent. The data were subjected to one-way analysis of variance (ANOVA), using the SPSS-17 software. *p*-Value less than 0.05 were regarded as significant.

Results

Antibacterial activity of 6 plants belonging to 5 botanical families was evaluated in vitro against 8 drug-resistant clinical isolates. The antimicrobial activity of the extracts and their potency was quantitatively assessed by the presence or absence of inhibition (Table 3). Maximum inhibition were observed with the extract of *M. comminus* L. against *S. aureus* (28 mm) and minimum inhibition were observed with the extract of *M. vulgari* against *Morganella morgani* (5 mm).

The least MIC and MBC value was observed by the ethanol extract of *M. comminus* L. against *S. aureus* (1.25 and 2.5 mg/mL) (Table 1). The levels of MIC and MBC for negative bacteria were observed ranges from 1.25 to 20 and 2.5 to 20 mg/mL in radius respectively (Table 3). The least MIC and MBC value was observed by the ethanol extract of *P. harmal* and *S. Montana* against *E. coli* (2.5 mg/mL) and ethanol extract of *M. vulgari* and *M. comminus* L. had minimum inhibitory effect on *M. morgani* (Table 3). In total, *S. Montana* extracts showed relatively same antibacterial activity against all bacteria and *M. morgani* was the more resistant bacteria for all plants extracts (Table 3).

Table 1. List of the studied plant

Studied plant	Family	Plant part
Marrubium vulgari	Lamiaceae	Leaf
Saturja montana	Lamiaceae	Leaf
Myrtus comminus L	Myrtaceae	Leaf
Amaranthus retriflexus	Amaranthaceae	Leaf
Cumminum cyminum L	Apiaceae	Seed
Peganum harmal	Zygophyllaceae	Seed

Table 2. The pattern antibiotic of isolation bacteria

Bacteria	Antibiotic-resistant
Staphylococcus aureus	Penicillin, oxacillin, trimethoprim, sulfamethoxazole
Staphylococcus saprophyticus	Ceftazidim, erythromycin
Staphylococcus epidermidis	Trimethoprim, sulfamethoxazole, erythromycin
Citrobacter freundii	Nalidixic acid
Pseudomonas aeruginosa	Nalidixic acid, ceftriaxon, cotrimoxazole, novobiocin
Morganella morgani	Ciprofloxacin, ceftriaxon, gentamicin, cotrimoxazole, nalidixic acid
Escherichia coli	Erythromycin, tetracycline
Enterobacter cloacae	Erythromycin tetracycline

Table 3. Antibacterial activity of the ethanolic extracts of plants against multi-drug resistant bacteria

Extract plant	M. vulgari (mm)	S. montana (mm)	M. comminus L. (mm)	A. retriflexus (mm)	P. harmal (mm)	C. cyminum L. (mm)
S. aureus	25	22	28	22	25	22
S. saprophyticus	23	18	19	14	24	19
S. epidermidis	13	16	14	11	15	14
C. freundii	10	13	12	0	15	10.5
P. aeruginosa	6	10	5	6.5	9.5	7
M. morgani	5	9	5.5	7	10	7
E. coli	6	15	12	13	17	10
E. cloacae	10	17	20	10	17	12

Table 4. MIC values of selected plant ethanol extracts

Extract plant	M. vulgari (mg/mL)	S. montana (mg/mL)	M. comminus L. (mg/mL)	A. retriflexus (mg/mL)	P. harmal (mg/mL)	C. cyminum L. (mg/mL)
S. aureus	2.5/5	5/10	1.25/2.5	5/10	2.5/5	5/10
S. saprophyticus	2.5/5	5/5	5/10	10/20	2.5/5	5/10
S. epidermidis	10/10	5/10	5/10	10/20	5/10	5/10
C. freundii	10/10	5/5	10/10	0	5/5	10/10
P. aeruginosa	10/20	5/10	10/20	10/20	5/5	10/10
M. morgani	20/20	5/10	20/20	10/20	5/10	10/20
E. coli	10/10	2.5/2.5	5/10	5/10	2.5/2.5	5/10
E. cloacae	5/10	2.5/5	1.25/2.5	5/10	2.5/2.5	2.5/5

Discussion

The results showed that the ethanol extract of leaf *M. vulgari*, *S. montana*, *M. comminus L.*, *A. retriflexus*, seed of *C. cyminum L.* and *P. harmal* had the best antimicrobial activity. The results revealed that the extract showed antibacterial activity with varying magnitudes, depending on the concentration of extract. A more significant inhibition was seen with a higher extract concentration. The least MIC and MBC value was observed by the ethanol extract of *M. comminus L.* against *S. aureus* (1.25 and 2.5 mg/mL) and the least MIC and MBC value was observed by the ethanol extract of *P. harmal* and *S. Montana* against *E. coli* (2.5 mg/mL). Ethanol extract of *M. vulgari* and *M. comminus L.* had minimum inhibitory effect on *M. morgani*.

Humans have frequently used plants to treat common infectious diseases, and some of these traditional medicines are still part of the habitual treatment of

various maladies [14]. Natural products and related drugs are used to treat 87% of all categorized human diseases including bacterial infection, cancer and immunological disorders [15]. Plants, as the source of medicine have been playing an important role in the health services around the globe [16]. Akin et al. also assayed antimicrobial activity of *M. comminus L.* against 7 pathogen bacteria (*S. aureus*, *Listeria monocytogenes*, *Enterococcus durans*, *Salmonella typhi*, *E. coli*, *P. aeruginosa* and *Bacillus subtilis*). It showed some activity on Gram-positive and Gram-negative bacteria [17]. Yadegarinia et al. reported excellent antimicrobial activities of *M. comminus L.* oil against *E. coli*, *S. aureus* and *C. albicans* [18]. The essential oil of Myrtle showed good antimicrobial activity against clinical strains of *Mycobacterium tuberculosis* [19]. The study of Mert et al. result show that n-hexane, methanol, ethanol, ethyl acetate and water extracts of *M. comminus L.* inhibited

the growth of *E. coli* ATCC 29998, *E. coli* ATCC 11230, *S. epidermidis* ATCC 12228, *S. typhimurium* CCM 5445 and *P. aeruginosa* ATCC 27853. The growth of *E. coli* ATCC 25922 was only inhibited by the methanol extract [20]. Recently, it has been reported that the essential oil of *M. communis* strongly active against *S. typhimurium* [20]. In the study, in total, *S. Montana* extracts showed relatively same antibacterial activity against all Gram-negative bacteria. The study of Djenane et al. the result show *S. montana* essential oil exhibited strong antimicrobial activity against *L. monocytogenes* [21].

The study of Adiguzel et al. the result show that the *S. montana* oil could not inhibit the growth of some bacteria including *B. subtilis*, *E. faecalis*, *E. coli*, *M. morgani*, *Proteus vulgaris*, *S. aureus* and *S. pyogenes* [22]. Many of the previous studies demonstrated that the members of the genus *Satureja* show a high antimicrobial activity due to the presence of thymol, carvacrol, and their precursors [23]. Moreover, *S. Montana* essential oil exhibited high antibacterial activities with strong effectiveness against several pathogenic food isolated *Salmonella* spp. including *S. enteritidis* with a diameter of inhibition zones growth ranging from 21 to 51 mm and MIC and MBC values ranging from 0.39-1.56 mg/mL to 0.39-3.12 mg/mL, respectively [24]. Our results demonstrate possible antibacterial effects of same components in *M. vulgari* and *P. harmal* on Gram-positive bacteria specially *S. aureus* and *S. saprophyticus*. The resistance of Gram-negative bacteria to plant extracts was not unexpected as; in general, this class of bacteria is more resistant than Gram-positive bacteria. Such resistance could be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism *S. montana* [25].

Darabpour et al. show that among Gram-positive bacteria, *S. epidermidis* and *S. pyogenes* were the most sensitive strains to the seed and root extracts while among Gram-negative bacteria, *K. pneumonia* and *B. melitensis* had the most sensitivity to the seed extract and *K. pneumonia* and *E. coli* to the root extract too [26]. The study of Abdollahi et al. *P. harmala* extract was observed to have antimicrobial activity against *E. coli* ATCC 25922 [27]. Butanolic and ethyl acetate extracts showed antibacterial activity against Gram-positive bacteria with MICs values ranging between 0.512 and 5 mg/mL but this activity is lower [28]. Its seed extracts of *P. harmal* are known to contain β -carboline alkaloids, anthraquinones, and a *S. montana* all quantity of flavonoid glycosides [29]. There are reports that alkaloids in *P. harmal* seed are mainly responsible for different pharmacological activities including antibacterial effects in addition to vasorelaxant, antihemosporean, anticancer,

antinociceptive, antitumor or antineoplastic and antiprotozoal effects.

The results of Abadi and Hassani demonstrated that the major components of the *M. vulgare* L. essential oil were: 4, 8, 12, 16-tetramethyl heptadecan-4-ol, germacrene D-4-ol, α -pinene, phytol, dehydro-sabina ketone, piperitone, δ -cadinene, 1-octen-3-ol and benzaldehyde [30]. The study of Bezi et al. oil of *S. Montana* show that *E. coli* was the most sensitive strain tested, with the strongest inhibition zones (23-32 mm), followed *C. albicans* with inhibition zones (21-28 mm). The methicillin-resistant *S. aureus* (MRSA), which causes many public health problems, was susceptible to winter savory oil, with an inhibition zone of 19-25 mm and the tested strains *B. subtilis*, *Serratia marcescens* and *Enterococcus faecium* were more sensitive to the oil of *S. montana*. *P. aeruginosa*, known to be very resistant even to synthetic drugs, exhibited weak inhibition zones (4-7 mm) [31]. The oil of *S. montana* is characterized by a high content of the phenolic monoterpene carvacrol. Other important compounds were the monoterpene hydrocarbons p-cymene, terpinene and the oxygen-containing compounds carvacrol methyl ether, borneol, thymol and thymol methyl ether.

The essential oil also contained *S. montana* percentages of α -thujene, α -terpinene, α -pinene and myrcene [31]. In conclusion, the incorporation of this extract into the drug formulations is also recommended. The results of this study present the herb as a good candidate to explore new alternative antibacterial agents to combat pathogenic microorganisms.

All of the plant extracts tested in this study had potential antibacterial activities against the reference strains. Our results support the use of these plants in traditional medicine and suggest that some of the plant extracts possess compounds with good antibacterial properties that can be used as antimicrobial agents in the search for new drugs.

Acknowledgements

This study was supported by Institute of Plant Biotechnology University of Zabol, Zabol, Iran. (Performer: Yasub Shiri).

Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

Funding/Support

Institute of Agriculture Research, University of Zabol.

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Please cite this article as: Shiri Y, Solouki M, Saeidi S. Activity of some Iranian plant extracts against multi-drug resistant human pathogens isolated from urinary tract infections. Zahedan J Res Med Sci. 2014; 16(10): 50-54.