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A survey on *Hibiscus rosa-sinensis*, *Alcea rosea* L. and *Malva neglecta* Wallr as antibacterial agents

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ABSTRACT

Objective: To guide for selection of plants with antibacterial activity for further phytochemical works on the isolation and identification of the active compounds. **Methods:** Ethanolic extracts of 3 species from *Malvaceae* family were evaluated by agar disc diffusion method for antibacterial activity against some gram-positive and gram-negative bacteria (*Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Bacillus cereus*, *Bacillus anthracis*, *Escherichia coli* and *Streptococcus pyogenes*). The extracts were obtained from aerial parts of *Hibiscus rosa* (*H. rosa*)-*sinensis* (leaf and flower), *Alcea rosea* (*A. rosea*) L. (leaf and flower) and *Malva neglecta* (*M. neglecta*) Wallr (flower). **Results:** These extracts had inhibitory effects at different concentrations (0.05, 0.10, 0.20 and 0.40 g/mL) against above mentioned bacteria. *Escherichia coli* was the most resistant strain. The highest inhibitory zone was showed by ethanolic extract of *M. neglecta* against *Staphylococcus epidermidis* (22 mm) and followed by ethanolic extract from flower of *H. rosa against Staphylococcus epidermidis* and *Staphylococcus aureus* (20 mm). The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values against *Staphylococcus epidermidis* were equal (MIC=MBC=5 mg/mL for *M. neglecta* extract and for *H. rosa* extract MIC=MBC=20 mg/mL). **Conclusions:** These findings suggest that these native plants have good antibacterial properties that can be used for infection control and treatment and could also be as new source for antibiotics discovery and infection treatment.

1. Introduction

The use of plants as a source of remedies for the treatment of many diseases dated back to prehistory and people of all continents have this old tradition[1]. In many parts of the world there is a rich tradition in the use of natural product for the treatment of many infectious diseases. Natural products have been used for thousands of years in traditional medicine for many purposes. Many herbal remedies have been used because of their anti-bacterial, anti-inflammatory, cytostatic, anti-fungal and anti-viral activities[2]. Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in developing world[3]. Traditional medicine is the oldest method of curing diseases and infections and various plants have been used in different parts of the world to treat human diseases and infections. Different parts of the plant have also been used for various forms of diseases and infections[4]. The medicinal plants employed in traditional medicine represent potential sources of cheap and effective standardized herbal medicines (phytomedicines) and novel molecules for the development of new chemotherapeutic agents[5]. Medicinal plants are known to owe their curative potentials from certain biological active substances, which exist in parts of the plants. The chemicals which are referred to as active or phytochemical substances include terpenses, flavonoid, bioflavonoid, benzophonones, xanthenes as well as some metabolites such as tannins, saponins, cyanates, oxalate and anthraxquinones[4]. In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. More than 13 000 plants have been studied during the last 5 year period[6].

The malvaceae family comprises about 120 genera and 1 700 to 2 000 species[7]. The species of this family, especially *Alcea rosea* (*A. rosea*) and *Malve neglecta* (*M. neglecta*) has been used as herbal plants in folk medicine for treatment of different diseases such as common cold and cough in

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Khuzestan, Iran. All parts of these plants are antiphlogistic, astringent, demulcent, diuretic, emollient, expectorant, laxative and salve. The leaves and flowers are the main used parts, their demulcent properties making them valuable as a poultice for bruise, inflammations, insect bites and etc, or taken internally for treatment of respiratory system diseases or inflammation of the digestive or urinary systems. Furthermore, it has been reported that *Hibiscus rosa* (*H. rosa*) (malvaceae) possesses anti-complementary, anti-diarrhetic and anti-phlogistic activity. *H. rosa* flowers have anti-spermatogenic, androgenic, anti-tumour and anti-convulsant activities[8].

In this study, in order to demonstrate the antimicrobial activity of these three plants (*A. rosea*, *M. neglecta* and *H. rosa-sinensis*), the effects of their ethanolic extracts were investigated against ten bacterial species.

2. Materials and methods

2.1. Plant material

The plant samples were collected from Shahid Chamran University farmlands in Khuzestan Province, Iran in June, 2009. The taxonomic identification of these plants was done comparing with existing herbarium in Biology Department of Shahid Chamran University.

2.2. Extract preparation

The aerial parts of these plants were dried in an oven at 40 ℃ and then powdered using electronic blender. The ethanolic extracts were prepared using 1 g of each plant powder and 10 mL of 80% ethanol (ethanol-distilled water, 8∶2 w/v). After that, the samples were centrifuged (3 000 rpm) for 15 min, and their supernatants were harvested and collected. This procedure was repeated three times. Eventually, the extracts were placed at room temperature in order to solvent evaporation.

2.3. Bacterial species

The ten bacterial species which used in this study were originally clinical isolates from patients. The gram-positive species were *Listeria monocytogenes* (*L. monocytogenes*), *Staphylococcus epidermidis* (*S. epidermidis*), *Staphylococcus aureus* (*S. aureus*), *Bacillus cereus* (*B. cereus*), *Bacillus anthracis* (*B. anthracis*), *Streptococcus pyogenes* (*Str. pyogenes*) and gram-negative species were *Pseudononas aeruginosa* (*P. aeruginosa*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Salmonella typhi* (*S. typhi*), and *Escherichia coli* (*E. coli*). They were identified according to standard phenotypic tests.

2.4. Determination of antibacterial activity

Four concentrations of each extract (0.05, 0.10, 0.20 and 0.40 g/mL) were prepared and their antibacterial activity was assessed by disc diffusion method against test bacteria. Stock culture of test bacteria were grown in nutrient broth medium at 37 ℃ for 22 h. Final bacterial number were adjusted to 0.5 McFarland turbidometry[9]. A lawn culture then prepared on Muller-Hinton agar (MHA, Merck) using sterile cotton swab. Sterile 6 mm filter paper discs[10] were placed on these cultures and impregnated with 50 μL volumes of the each concentration. The plates were left at room temperature for about 1 h to allow the diffusion of extracts from the discs into the medium, and were then incubated at 37 ℃ for 24 h. After incubation, the diameter of the zone of bacterial growth inhibition around each disc were measured and recorded in millimeter. Standard antibiotics including Nafcillin (NF) 1 mcg, Carbenicillin (CB) 100 mcg, Novobiocin (NB) 30 mcg, Doxycycline (DX) 30 mcg, Colistin (CL) 10 mcg, Methicillin (MT) 5 mcg, Oxacycline (OX) 1 mcg were used as controls for comparing the results. In order to determine the possible inhibitory effect of ethanol on test bacteria, discs containing 80% ethanol were also tested.

2.5. Determination of Minimum Inhibitory Concentration (MIC)

In order to determine MIC, a serial dilution of each extract (2.5, 5, 10, 20, 40, 50 and 80 mg/mL) was prepared. These dilutions were added to tubes containing 1 mL Muller Hinton broth and 30 μL of bacterial suspension was also added. The tubes were incubated at 37 ℃ for 24 h. The MIC of these extracts was determined for the most sensitive bacterial species. The lowest concentration of crude extract in broth medium that had inhibited the growth of the test microorganism was considered as MIC.

2.6. Determination of Minimum Bactericidal Concentration (MBC)

To determine the MBC, a loopful of broth from those tubes which did not exhibit any visible growth in the MIC assay was cultured on freshly prepared sterile Muller-Hinton agar and then incubated at 37 ℃ for 18-24 h. After incubation the highest dilution (least concentration) that inhibited colony formation on a solid medium was considered as MBC.

3. Results

The results of antimicrobial activities of these ethanolic extracts were presented in Table 1-3. This antibacterial activity was quantitatively determined by the presence or absence of inhibition zone around the discs containing extract. The results exhibited that *E. coli* was the most resistant strain among 10 used bacterial species. In the screening experiment, every three samples had the activities to depress *S. aureus* and *S. epidermidis* bacterial growth and this antibacterial activity parallelled to increasing of concentration. The highest activity (about 22 mm inhibition zone) was showed by *M. neglecta* against *S. epidermidis* (at 0.4 g/mL) and followed by ethanolic extract of *H. rosa* flower against *S. epidermidis* and *S. aureus* (20 mm)(Table 2 and 3). On the other hand, *B. cereus*, *S. typhi* and *K. pneumoniae* were resistant to *M. neglecta* and *H. rosa* extracts (except leaf extract of *H. rosa* at 0.4 g/mL), while *A. rosea* extract

Table 1
Inhibition zone (mm)* of *A. rosea* ethanolic extrac at various concentrations on some pathogenic bacteria.

Bacterial specie		Various concentrations of *A. rosea* ethanol extract(mg/mL)							
		Flower				Leaf			
		0.05	0.10	0.2 0	0.40	0.05	0.10	0.20	0.40
Gram-positive bacteria	*B. anthracis*	7	10	11	15	8	9	9	14
	B. cereus	R	R	R	9	R	R	8	10
	S. aureus	10	13	17	18	8	8	10	13
	S. epidermidis	9	10	15	19	R	9	10	13
	L. monocytogenes	R	R	R	7	R	R	7	10
	Str. pyogenes	8	9	10	13	8	8	9	11
Gram-negative bacteria	*E. coli*	R	R	R	R	R	R	R	R
	S. Typhi	R	R	7	8	R	R	R	8
	K. pneumoniae	7	7	9	14	7	8	10	12
	P. aeruginosa	R	8	9	13	R	R	R	R

R: Resistant, *(6 mm) diameter disc.

Table 2
Inhibition zone (mm)* of *H. rosa-sinensis* ethanolic extrac at various concentrations on some pathogenic bacteria.

Bacterial specie		Various concentrations of *H. rosa* ethanol extract (mg/mL)							
		Flower				Leaf			
		0.05	0.10	0.20	0.40	0.05	0.10	0.20	0.40
Gram-positive bacteria	*B. anthracis*	R	7	7	8	7	8	8	8
	B. cereus	R	R	R	R	R	R	R	R
	S. aureus	R	12	16	20	7	7	9	10
	S. epidermidis	12	12	15	20	7	10	12	15
	L. monocytogenes	R	R	R	R	R	R	R	R
	Str. pyogenes	9	12	15	17	R	R	R	R
Gram-negative bacteria	*E. coli*	R	R	R	R	R	R	R	R
	S. Typhi	R	R	R	R	R	R	R	R
	K. pneumoniae	R	R	R	R	R	R	R	8
	P. aeruginosa	R	R	12	15	8	8	8	8

R: Resistant, *(6 mm) diameter disc.

demonstrated inhibitory activity against these strains. *S. epidermidis* and *S. aureus* were the most susceptible species to the different concentrations of three ethanolic extracts.

Ethanol could not be as a factor that might affect these results, because the discs containing 80% ethanol did not have inhibition zone due to the volatile nature of ethanol. The inhibition zone of CB and DX against *S. aureus* compared to various concentrations of ethanolic extract of *H. rosa* (flower) was significant (Table 4).

The MIC and MBC values for *S. epidermidis* were equal (MIC=MBC=5 mg/mL for *M. neglecta* and MIC=MBC=20 mg/mL for *H. rosa*).

4. Discussion

As far as medicinal plants are concerned, the scientific researches have proved the real effectiveness of active principles in many plants, and there are not few species that should be still investigated under this point of view[11]. Ethnobotanical approach is one of the common methods that are employed in choosing the plants for pharmacological study[11]. By the end of the 19th century ethnobotany had started to develop as a science, providing a new tool for pharmaceutical research. Public institutions, such as the World Health Organization, and private pharmaceutical companies started to invest funds in ethnobotanical expeditions to tropical regions (mainly in America and Africa), to gather indigenous knowledge of medicinal plants and collect samples for laboratory investigation[13]. Medicinal herbs are the local heritage with global importance. Medicinal herbs have curative properties due to the presence of various complex chemical substance of different composition, which are found as secondary plant metabolites in one or more parts of these plants. These plant metabolites according to their composition are grouped as alkaloids, glycosides, corticosteroids, essential oil and etc.[14]. But genotypic variations need to be considered as needs seasonality or interannual variability in secondary metabolite production. Secondary metabolites may also be produced depending on the developmental stage of the plant[15].

There is a need to search for new antimicrobial agents because infectious diseases are still a global problem because of the development and spread of drug-resistant pathogens[16]. Plant extracts of many higher plants have

Table 3

Inhibition zone (mm)* of *M. neglecta*-Wallr flower ethanolic extrac at various concentrations on some pathogenic bacteria.

Bacterial specie		Various concentrations of *M. neglecta* (mg/mL)			
		0.05	0.10	0.20	0.40
Gram-positive bacteria	*B. anthracis*	8	8	8	10
	B. cereus	R	R	R	R
	S. aureus	8	9	11	13
	S. epidermidis	13	19	20	22
	L. monocytogenes	R	R	R	8
	Str. pyogenes	R	R	R	R
Gram-negative bacteria	*E. coli*	R	R	R	R
	S. typhi	R	R	R	R
	K. pneumoniae	R	R	R	R
	P. aeruginosa	7	8	8	10

R: Resistant, *(6 mm) diameter disc.

Table 4

Inhibition zone (mm)* of standard antibiotics on tested bacteria.

Bacterial specie		Antibiotic discs						
		NF	CB	NB	DX	CL	MT	OX
Gram-positive bacteria	*B. anthracis*	R	28	20	32	R	23	R
	B. cereus	R	7	18	18	R	R	R
	S. aureus	R	13	31	15	R	R	R
	S. epidermidis	R	36	29	21	R	R	R
	L. monocytogenes	25	19	28	20	12	R	R
	Str. Pyogenes	–	–	–	–	–	–	–
Gram-negative bacteria	*E. coli*	R	R	17	11	R	R	R
	S. Typhi	R	27	34	30	R	R	R
	K. pneumoiae	R	R	11	R	11	R	R
	P. aeruginosa	R	R	16	R	15	R	R

*(6 mm) diameter disc, R: Resistant, –: Not used.

been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trails[17]. It is obvious that the phytochemicals will find their way in the arsenal of antimicrobial drugs prescribed by physicians. Each antibiotic has a limited effective life and the public is becoming increasingly aware of problems with the over prescription of these antibiotics. In addition, many people, principally in the developing countries are interested in having more autonomy over their medical care, so self-medication is commonplace[1].

The resistance of bacteria to the enumerous antimicrobial agents constitutes one of the great challenges in the treatment of infections, condition that necessitate of searching and finding new sources of substances with antimicrobial proprieties to be used in the combat of microorganisms[7]. The study shows that extracts of tested plant show antibacterial activity against both gram-negative and gram-positive bacteria. In general, considering diameter of inhibition zones presented that the ethanolic extract of these plants is more effective against gram-positive bacteria than gram-negative bacteria and their antibacterial activity at lower concentrations is decreased. Antibacterial effect of *A. rosea* ethanolic extract against *K. pneumoniae* is valuable because this bacterium is a capsulated bacterium with high drug resistance and is an important causative agent for nosocomial infections. So, we can survey this extract against other capsulated bacteria such as *Streptococcus pneumoniae* whose resistant strains to penicillins and macrolides (the antibacterial agents used most frequently for pneumococcal infections) have became prevalent throughout the world[18]. In this study, all of tested plants had inhibitory effect against *P. aeruginosa* (as an opportunistic human pathogen) but their flower extract was more effective than the leaf extract.

S. aureus cause serious community-acquired and nosocomial infections. Methicillin-resistant *S. aureus* (MRSA) has increased and been cause of nosocomial infection problem in many countries[19]. *S. epidermidis* is the most common cause of nosocomial bacteremia and is the principal organism responsible for infections of implanted prosthetic medical devices such as prosthetic heart valves, artificial joints, and cerebrospinal fluid shunts. Infections caused by *S. epidermidis* are often persistent and relapsing[20]. In this study *M. neglecta* and *H. rosa* extracts did not show inhibitory activity against *B. cereus*, *S.* Typhi and *K. pneumoniae* (except leaf extract of *H. rosa* at 0.4 g/mL), while they had considerable effect against *S. epidermidis* and *S. aureus* and that was significant. In fact, in comparison to some standard antibiotics, *S. epidermidis* and *S. aureus* were

more susceptible to the extracts of *M. neglecta* and *H. rosa* than standard antibiotics. It is found that these extracts have higher antibacterial effects than some standard antibiotics against the bacterial cultures. The results demonstrate that *E. coli* exhibit resistance to all of tested plant extracts and *A. rosea* even dose not have inhibitory effect against it.This is in coordinate the results of Lee *et al* that showed *A. rosea* dose not have antibacterial activity against *E. coli*[21]. However, the solvent and the extraction system may both modify the final results and suitable solvent is important to get maximum antibacterial activity[22]. For example, it has been shown that methanolic extract of *Malva sylvestris* dose not show antimicrobial activity against *E. coli*, *S. epidermidis* and *S. aureus*, while ethanolic extract of it is active against *E. coli*, *Bacillus subtilis* and *P. aeruginosa*[23]. The MIC and MBC values for ethanolic extract of *M. neglecta* and *H. rosa* against *S. epidermidis* were the same; for bactericidal antimicrobials the MIC and MBC are often near or aquiline values[24], so it is concluded that ethanolic extracts of these plants have bactericidal effects on *S. epidermidis*.

The results of this study contribute to the scientific validation for the use of these medicinal plants in traditional medicine and serve as a guide for selection of plants with antibacterial activity for further phytochemical work on the isolation and identification of the active compounds.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1]Karou D, Dicko MH, Simpore J, Traore AS. Antioxidant and antibacterial activities of polyphenols from ethnomedicinal plants of Burkina Faso. *Afr J Biotechnol* 2005; **4**(8): 823–8.

[2]Rahim ZHA, Khan HBSG. Comparative studies on the effect of crude aqueous (CA) and solvent (CM) extracts of clove on the cariogenic properties of *Streptococcus mutans*. *J Oral Sci* 2006; **48**: 117–23.

[3]Igbinosa OO, Igbinosa EO, Aiyegoro OA. Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn). *Afr J Pharmacol* 2009; **3**(2): 58–62.

[4]Ekpo MA, Etim PC. Antimicrobial activity of ethanolic and aqueous extracts of *Sida acuta* on microorganisms from skin infections. *J Med Plants Res* 2009; **3**(9): 621–4.

[5]Ekundayo EO, Ezeogu LI. Evaluation of antimicrobial activities of extracts of five plants used in traditional medicine in Nigeria. *Int J Trop Med* 2006; **1**(2): 93–6.

[6]Dahanukar SA, Kulkarni RA, Rege NN. Pharmacology of medicinal plants and natural products. *Ind J Pharmacol* 2000; **32**: S81–118.

[7]Nunes XP, Maia GLDA, Almeida JRGDS, Pereira FDO, Lima EDO. Antimicrobial activity of the essential oil of *Sida cordifolia* L. *Brazil J Pharmacogn* 2006; **16**(Supl.): 642–4.

[8]Lans C. Ethnomedicines used in Trinidad and Tobago for reproductive problems. *J Ethnobiol Ethnomed* 2007; **3**: 13.

[9]Burt SA, Reinders RD. Antibacterial activity of selected plant essential oils against *Escherichia coli* O157: H7. *Lett Appl Microbiol* 2003; **36**: 162–7.

[10]Hsieh PC, Mau JL, Huang SH. Antimicrobial effect of various combinations of plant extracts. *Food Microbiol* 2001; **18**: 35–43.

[11]Menale B, Amato G, Prisco CD, Muoio R. Traditional uses of plants in North-Western Molise (central Italy). *Delpinoa* 2006; **48**: 29–36.

[12]Satish S, Raghavendra MP, Raveesha KA. Evaluation of antibacterial potential of some plants against human pathogenic bacteria. *Adv Biol Res* 2008; **2**(3–4): 44–8.

[13]Camejo-Rodrigues J, Ascensão L, Bonet MÀ, Vallès J. An ethnobotanical study of medicinal and aromatic plants in the Natural Park of "Serra de São Mamede" (Partugal). *J Ethnopharmacol* 2003; **89**: 199–209.

[14]Patil SB, Naikwade MNS, Magdum CS. Review on phytochemistry and pharmacological aspects of *Euphorbia hirta* Linn. *JPRHC* 2009; **1**: 113–33.

[15]Liebezeit G, Rau MT. New guinean mangroves-traditional usage and chemistry of natural products. *Senckenbergiana Maritima* 2006; **36**(1): 1–10.

[16]Wiyakrutta S, Sriubolmas N, Panphut W, Thongon N, Danwiset-Kanjana K, Ruangrungsi N, et al. Endophytic fungi with anti-microbial, anti-cancer and anti-malarial activities isolated from Thai medicinal plants. *World J Microbiol Biotechnol* 2004; **20**: 265–72.

[17]Satish S, Mohana DC, Raghavendra MP, Raveesha KA. Antifungal activity of some plant extracts against important seed borne pathogens of *Aspergillus* sp. *J Agr Technol* 2007; **3**(1): 109–19.

[18]Stratton CW. Dead bugs don't mutate: susceptibility issues in the emergence of bacterial resistance. *Em Inf Dis* 2003; **9**: 10–6.

[19]Sittiwet C, Jesadanont S, Pongpech P, Naenna P, Pongsamart S. Antibacterial activity of *Malvastrum coromandelianum* garcke against methicillin-sensitive and methicillin-resistant strains of *Staphylococcus aureus*. *Curr Res Bacteriol* 2008; **1**(1): 42–5.

[20]Parekh J, Chanda SV. Antibacterial activity of aqueous and alcoholic extracts of 34 Indian medicinal plants against some *Staphylococcus* species. *Turk J Biol* 2008; **32**: 63–71.

[21]Lee J, Lee J, Lim J, Sim S, Park D. Antibacterial effects of S-(-)-tulipalin B isolated from *Spiraea thunbergii* Sieb. on *Escherichia coli*, a major food borne pathogenic microorganism. *J Med Plants Res* 2008; **2**(3): 59–65.

[22]Muthuvelan B, Balaji-Raja R. Studies on the efficiency of different extraction procedures on the anti microbial activity of selected medicinal plants. *World J Microbiol Biotechnol* 2008; **24**: 2837–42.

[23]Souza GCD, Haas APS, Poser GLV, Schapoval EES, Elisabetsky E. Ethnopharmacological studies of antimicrobial remedies in the south of Brazil. *J Ethnopharmacol* 2004; **90**: 135–43.

[24]Reuben KD, Abdulrahman FI, Akan JC, Usman H, Sodipo OA, Egwu GO. Phytochemical screening and in vitro antimicrobial investigation of the methanolic extract of *Croton zambesicus* Muell Arg. stem bark. *Eur J Scic Res* 2008; **23**: 134–40.