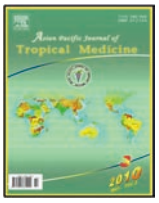
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm



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Preliminary study on the antibacterial activity of some medicinal plants of Khuzestan (Iran)

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ARTICLE INFO

Article history:
Received 21 December 2009
Received in revised form 20 January 2010
Accepted 20 February 2010
Available online 20 March 2010

Keywords:
Antibacterial activity
Amaranthus graecizans
Beta vulgaris
Polygonum patulum
Rumex obtusifolius
Ethanolic extract

ABSTRACT

Objective: To search for antimicrobial agents among natural products. **Methods:** Ethanolic extracts of 4 plant species, including *Beta vulgaris* L. (Chenopodiaceae), *Amaranthus graecizans* (*A. graecizans*) L. (Amaranthaceae), *Rumex obtusifolius* (*R. obtusifolius*) L. and *Polygonum patulum* (*P. patulum*) M.B. (Polygonaceae), were evaluated for antibacterial activity using agar disc diffusion method against some gram-positive and gram-negative bacteria [*Pseudomonas aeruginosa* (*P. aeruginosa*), *Listeria monocytogenes* (*L. monocytogenes*), *Staphylococcus epidermidis* (*S. epidermidis*), *Staphylococcus aureus* (*S. aureus*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Salmonella typhi* (*S. typhi*), *Bacillus cereus* (*B. cereus*), *Bacillus anthracis* (*B. anthracis*), *Escherichia coli* (*E. coli*) and *Streptococcus pyogenes* (*Str. pyogenes*)]. These extracts were obtained from aerial parts of the used plants. **Results:** The majority of these extracts had inhibitory effect at different concentrations (0.05 g/mL, 0.10 g/mL, 0.20 g/mL and 0.40 g/mL) against above mentioned bacteria. *E. coli* was the most resistant strain. The highest inhibitory zone was showed by ethanolic extract of *P. patulum* against *Str. pyogenes* (28 mm) and followed by ethanolic extract of *B. vulgaris* against *S. epidermidis* (23 mm). The extract of *A. graecizans* didn't show inhibitory activity except at 0.40 g/mL against *B. cereus*. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of *R. obtusifolius* extract that was measured against *Str. pyogenes* were equal (MIC=MBC=5.00mg/mL). **Conclusion:** The findings of this study could also be as new source for antibiotics discovery and infection treatment.

1. Introduction

Traditional uses of plants for medicinal purposes provide a basis for the use of specific plants for specific medicinal conditions[1]. Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in developing world[2]. Historically, all medicinal preparations were derived from plants, whether of raw plant materials or of refined crude extracts, mixtures, etc. Recent estimates suggest that several thousands of plants have been known with medicinal applications in various cultures[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 plants have also been used for various forms of diseases and infections[4]. 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[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 principles or phytochemical substances include terpenses, flavonoids, bioflavonoids, benzophonones, xanthenes as well as some metabolites such as tannins, saponins, cyanates, oxalate and anthraxquinones[4]. In recent times, plant research has increased all over the world and a large number 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 Polygonaceae family comprises approximately 40 genera and 800 species located in tropical, subtropical

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and temperate regions[7]. Plants belonging to this family are known to produce a large number of biologically important secondary metabolites, such as flavonoids, anthraquinones, alkaloids and steroids[8]. *Rumex* and *Polygonum* belonged to this family. The genus Polygonum comprises of about 150 species. The presence of diverse secondary metabolites like flavonoids, anthraquinones, phenylpropanoids and proanthocyanidins, has been reported from species of the genus *Polygonum*[9]. Leaves of some species of *Rumex* have been used for many diseases in traditional medicine, also in bacterial dysentery[10]. The "milk" of *Rumex obtusifolius* (*R. obtusifolius*) leaf is known to contain tannins and oxalic acid, which is an astringent. Members of Amaranthaceae family are good natural sources of carotenoids, vitamin C, nutritionally critical lysine, methionine and proteins[11]. The Chenopodiaceae family has probably about 100 genera and 1 400 species that are mainly in arid areas, deserts and coastal and saline habitats of North and South Africa, Asia, Australia, Europe and North and South America.

In this study, in order to demonstrate the antimicrobial activity of these four plants [*Beta vulgaris* (*B. vulgaris*), *Amaranthus graecizans* (*A. graecizans*), *R. obtusifolius* and *Polygonum patulum* (*P. patulum*)], the effects of their ethanolic extracts were investigated against 10 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 mins, and their supernatants were harvested. 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 *Pseudomonas 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 g/mL, 0.10 g/mL, 0.20 g/mL 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 Mc Farland turbidometry[12]. A lawn culture then prepared on Muller-Hinton agar (MHA, Merck) using sterile cotton swab. Sterile 6 mm filter paper discs[13] were placed on these cultures and impregnated with 50 μL of each concentration. The plates were left at room temperature for about 1 h to allow the extract to diffuse 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 1 mcg, Carbenicillin 100 mcg, Novobiocin 30 mcg, Doxycycline 30 mcg, Colistin 10 mcg, Methicillin 5 mcg and Oxacycline 1 mcg were used as controls for comparing the results. In order to determination the possible inhibitory effect of ethanol on test bacteria, discs containing 80% ethanol were also tested.

2.5. Determination of minimum inhibitory concentration

In order to determining minimum inhibitory concentration (MIC), a serial dilution of each extract (2.5 mg/mL, 5.0 mg/mL, 10.0 mg/mL, 20.0 mg/mL, 40.0 mg/mL, 50.0 mg/mL 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 the extract 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

To determine the minimum bactericidal concentration (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 the ethanolic extracts of these plants are presented in Table 1&2. 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* and

S. typhi were the most resistant strains among 10 tested bacterial species and followed by *K. pneumoniae* and *P. aeruginosa*, respectively. In fact, all of the used extracts had not significant antibacterial effects against gram-negative bacteria. Among four plant samples, *A. graecizans* ethanolic extract showed no inhibitory activity at all, except against *B. cereus* at 0.40 g/mL. In this screening experiment, *P. patulum* and *R. obtusifolius* had considerable effect to suppress *Str. pyogenes* bacterial growth and this antibacterial activity was increased parallel to increase in concentration. The highest activity (about 28 mm inhibition zone) was showed by *P. patulum* against *Str. pyogenes* (at 0.40 g/mL) and followed by ethanolic extract of *B. vulgaris* against *S. epidermidis* (23 mm) (Table 1 and 2). On the other hand, *B. cereus*, and *L. monocytogenes* were nearly resistant to *B. vulgaris* and *A. graecizans* extracts (except at 0.40 g/mL), while *P. patulum* and *R. obtusifolius* extracts demonstrated significant inhibitory activity against these strains. *Str. pyogenes* and *S. epidermidis* were the most susceptible species to the different concentrations of the ethanolic extracts of *P. patulum*, *R. obtusifolius* and *B. vulgaris*, respectively.

Ethanol could not be as a factor that might affect these results, because the discs containing 80% ethanol did not have zone of inhibition 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 *B. vulgaris* was significant (Table 2, 3).

It showed the obtained MIC and MBC values of *R. obtusifolius* extract for *Str. pyogenes* were equal (MIC=MBC=5.00 mg/mL). The MIC and MBC value of *P. patulum* extract for *Str. pyogenes* were 2.50 mg/mL and 5.00 mg/mL, respectively. And the MIC and MBC value of *B. vulgaris* extract for *S. epidermidis* were 20.00 mg/mL and 40.00 mg/mL, respectively.

Table 1

Inhibition zone (mm)* of *R. obtusifolius* and *P. patulum* ethanolic extracs at variou concentrations on tested bacteria.

Bacterial Sp.		*R. obtusifolius* (g/mL)				*P. patulum* (g/mL)			
		0.05	0.10	0.20	0.40	0.05	0.10	0.20	0.40
Gram-positive bacteria	*B. anthracis*	7	8	10	11	8	9	11	13
	B. cereus	8	9	9	10	8	9	11	11
	S. aureus	7	7	7	9	9	12	12	13
	S. epidermidis	10	11	13	13	10	16	19	17
	L. monocytogenes	8	9	9	10	8	9	11	13
	Str. pyogenes	10	13	12	16	15	22	25	28
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	R
	P. aeruginosa	R	R	R	R	R	R	R	R

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

Table 2

Inhibition zone (mm)* of *B. vulgaris* and *A. graecizans* ethanolic extracs at variou concentrations on tested bacteria.

Bacterial Sp.		*B. vulgaris* (g/mL)				*A. graecizans* (g/mL)			
		0.05	0.10	0.20	0.40	0.05	0.10	0.20	0.40
Gram-positive bacteria	*B. anthracis*	R	7	9	9	R	R	R	R
	B. cereus	R	R	R	8	R	R	R	9
	S. aureus	8	11	13	15	R	R	R	R
	S. epidermidis	12	15	23	20	R	R	R	R
	L. monocytogenes	R	R	R	8	R	R	R	R
	Str. pyogenes	R	7	8	11	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	7	8	R	R	R	R
	P. aeruginosa	R	R	7	9	R	R	R	R

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

Table 3
Inhibition zone (mm)* of standard antibiotics on tested bacteria.

Bacterial Sp.		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

NF: Nafcillin 1 mcg, CB: Carbenicillin 100 mcg, NB: Novobiocin 30 mcg, DX: Doxycycline 30 mcg, CL: Colistin 10 mcg, MT: Methicillin 5 mcg, OX: Oxacycline 1 mcg, *(6mm) diameter disc, R: Resistant, –: Not used.

4. Discussion

Medical plants have been remedies for human disease for a long time because they contain components of therapeutic value[14]. Traditionally used medicinal plants produce a variety of compounds with known therapeutic properties. The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered candidates for developing new antimicrobial drugs[15]. 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[16]. Secondary metabolites play a role in the medicinal properties of plants[17].

There is continuous and urgent need for discovery of new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and reemerging infectious diseases[18]. Natural products are known to play an important role in both drug discovery and chemical biology. In fact, many of the current drugs either mimic naturally occurring molecules or have structures that are fully or in part derived from natural motifs. Natural antimicrobials can be derived from barks, stems, leaves, flowers and fruits of plants[19]. Scientific strategies for the *in vitro* evaluation of natural products with biological activity have changed in the past few years[20]. The search for newer sources of antibiotics is a global challenge, preoccupying research institutions, pharmaceutical companies and academia, since many infectious agents are becoming resistant to synthetic drugs[21]. The resistance of bacteria to the enumerous antimicrobial agents constitutes one of the greatest challenges in the treatment of infections under the condition that necessitate of searching and finding new sources of substances with antimicrobial proprieties to be used in the combat of microorganisms[22]. *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[18]. In this study *B. vulgaris* and *P. patulum* extracts did not show considerable inhibitory activity on gram-negative bacteria, while they had antibacterial effect against *S. epidermidis* that is significant. In fact in comparison to some standard antibiotics (such as NF and CL), *S. epidermidis* was more susceptible to the extracts of *B. vulgaris* and *P. patulum* than standard antibiotics. It was found that these extracts have higher antibacterial effect than some standard antibiotics against the tested species. Inhibitory activity of *P. patulum* against *B. anthracis* and *S. epidermidis* is similar with the results of Kazmi *et al* that showed the antibacterial effects of *P. equisetiforme* against *B. anthracis* and *S. epidermidis*[23]. The results of this study showed that *B. vulgaris* extract had not any antibacterial activity against *E. coli*, which is opposite with the results of Winkler *et al* that showed the extract of red beetroot *B. vulgaris* had antibacterial activity on *E. coli*[24]. However, *A. graecizans* and *B. vulgaris* are belonging to the same order, but in spite of *B. vulgaris*, *A. graecizans* had not any antibacterial activity against test strains. The results demonstrated that *E. coli* exhibited resistance to all of the tested plants. However, the solvent and the extraction system may both modify the final results and suitable solvent is important to get maximum antibacterial activity[25]. For example, Babu *et al* showed that the antibacterial activity is more significant in solvent extracts as compared to aqueous extract in all the plants indicating that the active principle responsible for

antibacterial activity is more soluble in organic solvents[26].

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.

Acknowledgements

The authors acknowledged Dr. Vaezi from Shahid Chamran University for the taxonomic identification of the plants that studied in this research and also for the vice chancellor of Shahid Chamran University for preparing research grant.

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