

ANTIMICROBIAL POTENTIAL OF THE LEAVES, BRANCHES AND PEELS OF SOME MEDICINAL PLANTS AGAINST VARIOUS PATHOGENIC MICROORGANISMS

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Abstract

Antimicrobial activity of medicinal plants is of great importance in the pharmaceutical industries as the active compounds present in the plants are the source for the synthesis of new drugs. In this study, four ethanolic extracts in the concentration of 5, 10 and 20 mg/ mL were prepared from *Allium cepa*, *Polyalthia longifolia* and *Punica granatum* plant parts. These extracts were tested against five different pathogenic cultures. All the four extracts showed significant activity against the tested pathogens with variable efficacies. Among the tested medicinal plants, *Punica granatum* peels and *Allium cepa* were found to be effective against all pathogenic bacteria. Results showed that *Pseudomonas aeruginosa* was inhibited by the extracts of *Punica granatum* peels at all three concentrations. Moreover, *Klebsiella* and *E. coli* were also inhibited by the extract prepared from branches of *Polyalthia longifolia* at all tested concentrations while the leaves extract of *Polyalthia longifolia* inhibit the pathogenic cultures at the concentration of 20 mg/ mL.

Introduction

Medicinal plants are rich source of compounds that are antimicrobial in nature. These plants are the source of new potential drugs. They may contain compounds that have anti inflammatory, healing, antidiabetic, anticancer, antioxidant as well as antimicrobial activities (Arun and Sing, 2012).

Punica granatum (Pomegranate) is one of the fruit whose peels and seeds possess various medicinal properties (Rahimi *et al.*, 2012). Various phytochemicals such as alkaloids, flavonoids and steroids of great medicinal importance are also present in *Allium cepa* (Onion). Most of the phytochemicals inhibit the growth of pathogenic bacteria (Bakht *et al.*, 2013). *Polyalthia longifolia* (False ashoka) is widely present in various regions of Pakistan. It can be a potential nutraceutical. It has been widely used in folk system of medicine (Mahajan, 2015).

Several pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Klebsiella* species are responsible for many nosocomial and non nosocomial infections in humans. Among these infections gastrointestinal tract infections and urinary tract infections are the most common (Nazir and Latif, 2012). As these strains adopt the resistance to the routinely used antibiotics, they may cause prolong illness in patients (Ifeanyichukwu *et al.*, 2015). Due to the increasing number of multi drug resistant microbial strains and high cost of synthetic antibiotics, it is necessary to evaluate the natural source of drugs to be used as infectious controlling agents. In this regard, the present study was carried out to investigate different parts of the medicinal plants to determine their antimicrobial potential against microbial infections.

Materials and Methods

Collection of bacterial cultures

Pure cultures of *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Klebsiella* species were collected from Dr. Essa Laboratory, North Nazimabad, Karachi.

Selection of plants for antimicrobial activity

Three medicinal plants *Allium cepa*, *Polyalthia longifolia* and *Punica granatum* were selected to screen their antimicrobial effects. Fresh and disease free leaves and branches of *Polyalthia longifolia* were collected from the Botanical garden of Jinnah University for Women, Karachi. Healthy peels of *Punica granatum* was collected from fresh juice center in Nazimabad, Karachi.

Preparation of the ethanolic extract of the selected plants

The selected parts of the plants were washed thoroughly with sterile deionized water. After washing the leaves, branches and peels were shade dried at room temperature. Dried parts were grounded to a powder form and soaked in 80% (v/v) ethanol for 2 days at room temperature. Extracts obtained from this method were filtered and concentrated by rotary vacuum evaporator. Dried extracts were weighed and stored at 4°C until used.

Antimicrobial activity of the ethanolic extract of selected plants

Antimicrobial activity of selected plants was determined as described by Valgas *et al.* (2007). All bacterial cultures were inoculated in nutrient broth and incubated at 37°C for 24 h. After incubation a loop full of every culture was inoculated on sterile nutrient agar plates separately. Plant extracts were diluted in sterile deionized water in order to obtain the final concentrations of 5, 10 and 20 mg/ mL. Four wells were made on nutrient agar plates with the help of sterile borer. The above three concentrations of each extract were placed in three wells while sterile deionized water was placed in last well as a negative control. All plates were incubated at 37°C for 24 h. After incubation, the zone of inhibition of bacterial cultures by different extracts were observed and measured in millimeters (mm).

Results and Discussion

Various pathogenic isolates were collected from the diagnostic lab. The sources of these cultures are listed in Table 1. Resistance or sensitivity of these pure cultures was tested for the extracts of *Punica granatum* peels, *Allium cepa*, and *Polyalthia longifolia*.

Punica granatum (Pomegranate) has wide therapeutic applications. Because of its medicinal properties, it is widely used in Ayurvedic and Unani medicines for the treatment of diarrhea, ulcers and diabetes. It has been reported that this fruit acts as a blood tonic and a good antiparasitic agent. Different parts of *Punica granatum* like bark, fruit, seeds and leave have great therapeutic values (Miguel *et al.*, 2010). In this study, we collected the peels of *Punica granatum* and the extracts of these peels were studied for antimicrobial properties. Results showed that the *Punica granatum* peels exhibit maximum zone of bacterial inhibition among the tested plants. As shown in Table 2, extracts of *Punica granatum* possess potential antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Klebsiella* species. The highest *Punica granatum* peels activity was recorded against *Pseudomonas aeruginosa* at the concentration of 5 mg/ mL while other cultures are inhibited at higher concentration. These bacterial cultures would be considered as dangerous pathogens and involved in extremely severe infections. Therefore, the present study suggested *Punica granatum* peels as an effective inhibitor of these pathogenic bacteria at a concentration of 20 mg/ mL.

Allium cepa (Onion) is an essential ingredient of daily consumed foods in Pakistan. It has great medicinal potential. Beside its hypolipidemic activity it is also very useful for its hepatoprotective as well as antibacterial activities (Sohail *et al.*, 2011). In the present study, the antibacterial activity of *Allium cepa* was checked against various pathogens isolated from various pus and urine samples. According to our analysis *Allium cepa* peels was found to significantly inhibit the tested pathogenic bacteria. Maximum inhibition of *Proteus mirabilis* and *Klebsiella* species were observed at the concentration of 20 mg/ mL (Table 3).

Polyalthia longifolia can be the main source of novel therapeutic agents. In this study antimicrobial activity of the leaves and branches of *Polyalthia longifolia* was observed against *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella* species (Table 4). *Escherichia coli* is one of the most common antibiotic resistant organisms involved in urinary tract infections (Croxall *et al.*, 2011). The results of the present study showed that the growth of *Escherichia coli* isolated from patient's urine and *Klebsiella* sp. were significantly inhibited by the branches of *Polyalthia longifolia* at the concentration of 5 mg/ mL. Other cultures were not inhibited by the branch extracts but *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella* species were inhibited by the leaves extract of the *Polyalthia longifolia* at the concentration of 20 mg/ mL. Therefore the extracts of leaves and branches *Polyalthia longifolia* possess significant antibacterial activities besides its analgesic and antiinflammatory potential as reported earlier (Jothy *et al.*, 2013; Dhimen *et al.*, 2016).

This study of the plant extracts demonstrated that the active compounds present in these extracts, were responsible for the inhibition of the growth of various pathogenic cultures. Further studies will focus on the isolation and biochemical characterization of these active compounds.

Table 1. Bacteria cultures and their source of isolation.

Bacterial Cultures	Site of isolation
<i>Escherichia coli</i>	Urine
<i>Escherichia coli</i>	Ascitic fluid
<i>Escherichia coli</i>	Pus
<i>Pseudomonas aeruginosa</i>	Urine
<i>Proteus mirabilis</i>	Pus
<i>Klebsiella</i>	Urine

Table 2. Antimicrobial activity of *Punica granatum* peels.

Strains	Zone of inhibition (mm)		
	5 mg/ mL	10 mg/ mL	20 mg/ mL
<i>Escherichia coli</i> (Urine)	0	4	4
<i>Escherichia coli</i> (Ascitic fluid)	0	0	5
<i>Escherichia coli</i> (Pus)	0	3	3
<i>Pseudomonas aeruginosa</i>	3	7	8
<i>Proteus mirabilis</i>	0	4	5
<i>Klebsiella</i>	0	5	7

Table 3. Antimicrobial activity of *Allium cepa*.

Strains	Zone of inhibition (mm)		
	5 mg/ mL	10 mg/ mL	20 mg/ mL
<i>Escherichia coli</i> (Urine)	0	1	2
<i>Escherichia coli</i> (Ascitic fluid)	0	1	3
<i>Escherichia coli</i> (Pus)	0	0	2
<i>Pseudomonas aeruginosa</i>	0	0	2
<i>Proteus mirabilis</i>	0	1	4
<i>Klebsiella</i>	0	2	4

Table 4. Antimicrobial activity of *Polyalthia longifolia*.

Strains	Zone of inhibition (mm)					
	Branches of <i>Polyalthia longifolia</i>			Leaves of <i>Polyalthia longifolia</i>		
	5 mg/mL	10 mg/mL	20 mg/mL	5 mg/mL	10 mg/mL	20 mg/mL
<i>Escherichia coli</i> (Urine)	3	6	5	0	3	4
<i>Escherichia coli</i> (Ascitic fluid)	0	0	0	0	0	0
<i>Escherichia coli</i> (Pus)	0	0	0	0	0	0
<i>Pseudomonas aeruginosa</i>	0	0	0	0	3	3
<i>Proteus mirabilis</i>	0	0	0	0	0	0
<i>Klebsiella</i>	3	4	4	0	0	4

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