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Antibacterial, antifungal and phytochemical analysis of selected medicinal plants

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ABSTRACT

Plants have potent components of phytomedicine. Plant based natural constituents can be derived from any part of the plant like bark, leaves, fruits, flowers, roots, seeds etc., The therapeutic use of medicinal plant is becoming popular because of its inability to cause side effects and combat antibiotic resistant microorganisms. Medicinal plants such as Psidium guajava, Punica granatum, Prosopsi juliflora and Aegle marmelos are important sources of antibacterial, antifungal and antioxidant compounds. These plants contain secondary metabolites such as alkaloids, flavonoids, steroids, phenolics, terpenes, volatile oils etc., These are important for radical scavenging effects as well as their potential antibacterial, estrogenic and anti- cancer activities. The objective of this research is to determine the antibacterial, antifungal and phytochemical analyses of the four medicinal plants. Punica granatum was more effective followed by Prosopis juliflora, Psidium guajava and Aegle marmelos.

Key words: Antibacterial activity, *Psidium guajava, Punica granatum, , Prosopsi juliflora , Aegle marmelos*

INTRODUCTION

Medicinal plants are rich sources of antimicrobial agents. Plants are used medicinally in different countries and are the source of potential and powerful drugs[1]. According to World health organization (WHO) more than 80% of the world population relies on traditional medicine for their primary health care Needs [2]. The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China, India and the north east, but it is thoughtless as art as old as mankind[3]. The potential of higher plants as a source for new drugs is still largely unexplored.

In last five decades, these plants have been extensively studied by advanced scientific techniques and reported for various medicinal properties *viz*, anticancer activity, antibacterial activity, antifungal activity, antidiabetic activity, antioxidant activity, hepatoprotective activity, haemolytic activity, larvicidal activity and anti-inflammatory activity [4,5]. Hence in this study, the antibacterial, antifungal activity and phytochemical analyses of selected medicinal plants were tested against human pathogens.

METERIALS AND METHODS

Collection of plant material

The medicinal plants used in this study were peels of *Puncia granatum*, and leaves of *Psidium guajava, Prosopis juliflora* and *Aegle marmelos*. Leaves and peels were collected from Coimbatore. The samples were collected in sterile polyethylene bags. The leaves were surface sterilized by using sodium hypochlorite and rinsed twice with distilled water[6].

Preparation of plant extracts

The fresh leaves were dried under shade. The dried plant material were powdered and extracted with methanol using soxhlet apparatus method. All the extracts were poured into sterile dry petriplates and the solvent was evaporated. The sediments were scrapped off, weighed and dissolved in DMSO.

Test organisms

Human pathogenic organisms (bacteria) isolated from clinical samples were used in this study. The organisms used were *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus cereus, Salmonella typhi, and Shigella dysentriae.* Fungal strains like *Candida albicans* (clinical isolate), *Mucor rouxii* (MTCC 386), *Penicillium citrenigrum* (MTCC 160), *Aspergillus niger* (MTCC 281), and *Fusarium oxysporium* (MTCC 284) were used.

Antimicrobial activity by Agar well diffusion method

The antimicrobial activity of the plant extracts were determined by agar well diffusion method. The inoculum of microorganisms was adjusted to 0.5 McFarland standard. About 15 to 20ml of Muller Hinton agar medium was poured in the sterilized petridish and allowed to solidify. Wells of 6mm in diameter and about 2cm apart were punctured in the culture medium. From each extract (1µl, 2µl, 3µl and 4µl) for bacterial and (20,30,40and50µl) for fungi were added to the well. Plates were incubated at 37^0C for 24 hours. Antimicrobial activities were evaluated by measuring inhibition zone diameters. Gentamicin and Amphotericin B were used as controls.

Minimum bactericidal Concentration (MBC)

The MBC was defined as lowest concentration able to kill any microbe. Dilutions of the plant extract were prepared in sterile nutrient broth to get a final concentration of 2 mg, 4 mg, 8 mg, 16 mg, 32 mg and 64 mg/ml respectively [7]. To each of these dilutions, a loop full of culture adjusted to 0.5 McFarland standard, was inoculated and all the tubes were incubated at 37^0c for 24 hrs. After incubation, loopful from each tube was inoculated onto nutrient agar plates. The plate without growth was recorded as MBC.

Phytochemical screening

Phytochemical screening for flavonoids, alkaloids, tannins, saponins and terpenoids were done following standard methods as described by different authors [8,9,10].

RESULTS AND DISCUSSION

The antibacterial activities of the extracts were shown in Table 1. Methanol extracts of all the plants were effective. *Salmonella typhi* was not affected by *Prosopis juliflora*. *Staphylococcus aureus was* inhibited to a greater extent by *Prosopis juliflora* than other extracts. *Punica granatum* showed excellent antibacterial activity towards all organisms tested. The zone diameter for plant extrcats were greater than the standard antibiotic gentamicin. *Aegle marmelos*, was effective at higher concentrations. Next to *Punica granatum* , *Prosopis juliflora* leaves had greater antibacterial activity. Towards fungi also, the *Punica granatum* extracts were highly effective. *Aspergillus niger* produced a wide zone of 37mm. The antifungal activities of the extracts were shown in Table.2. Minimum bactericidal concentration of *Punica granatum*, *Psidium guajava, Prosopis juliflora* and *Aegle marmelos*

Punica granatum commonly named as pomegranate is widely reported to possess several activities[11-18]. Various authors had reported the presence of a wide range of phytochemicals from *Punica granatum* [19,18, 20]. Mostafa *et al*. [21], had reported the antifungal activity of *Punica granatum* against *Aspergillus flavus*. They suggest these extracts as eco-friendly fungicides. The minimum bactericidal concentration of *Punica granatum* ranged from 4.0mg to 16mg/ml. The Minimum bactericidal concentration is low, thus showing good antibacterial activity against the tested organisms. The MBC of all the extracts were shown in table.3.

According to Sineenat siri *et al*. [22], the MIC of *Punica granatum* extract was 3.125mg/ml and MBC was 6.25 mg/ml. Our results are similar to these reports. Thelma *et al*., [23], had reported an MIC of 61.5 µg /ml for punicalagin and Burapadaja and Bunchoo [24], had found a MIC value of 768µg/ml for punicalagin.

Table.1. Antibacterial activity of methanol extracts of different plant extracts

S. NO	ORGANISMS	Zone of inhibition in mm																	
		Punica granatum				*Psidium guajava*				*Prosopis juliflora*				*Aegle marmelos*				CONTROL	
		1µl	2µl	3µl	4µl	1µl	2µl	3µl	4µl	1µl	2µl	3µl	4µl	20µl	30µl	40µl	50µl	Gentamicin	DMSO
1.	*Staphylococcus aureus*	12	14	15	17	11	12	13	14	16	22	23	25	15	16	16	17	14	-
2.	*Klebsiella pneumonia*	12	13	14	15	11	12	13	14	14	15	16	17	16	17	18	19	11	-
3.	*Bacillus subtilis*	12	14	15	16	12	13	14	15	12	13	14	15	15	16	17	18	10	-
4.	*Salmonella typhi*	11	12	13	14	10	10	11	12	-	-	-	-	11	12	13	15	10	-
5.	*Shigella dysenteriae*	13	13	14	15	11	12	14	15	-	-	-	-	12	13	14	15	10	-
6.	*Escherichia coli*	16	17	19	21	10	12	13	14	12	13	14	15	15	16	17	18	11	-
7.	*Pseudomonas aeruginosa*	13	14	15	15	9	10	10	11	4	5	6	7	12	13	14	15	14	-

Table. 2. Antifungal activity of methanol extracts of different plant extracts

S.NO	ORGANISMS	Zone of inhibition in mm																	
		Punica granatum				*Psidium guajava*				*Prosopis juliflora*				*Aegle marmelos*				*Control*	
		25µl	50µl	75µl	100µl	25µl	50µl	75µl	100µl	25µl	50µl	75µl	100µl	25µl	50µl	75µl	100µl	Amphotericin B	DMSO
1.	*Candida lbicans*	23	25	27	30	19	22	24	25	18	20	22	24	12	13	15	17	18	-
2.	*Mucor rouxii*	24	26	28	30	14	17	19	20	24	26	30	31	15	17	19	21	19	-
3.	*Penicillium citrenigrum*	28	30	32	34	17	18	21	22	25	27	30	32	12	14	16	17	19	-
4.	*Aspergillus niger*	30	33	35	37	22	25	27	29	24	26	28	30	6	8	10	12	14	-
5.	*Fusarium oxysporum*	25	27	29	31	17	19	21	23	11	13	15	17	-	-	-	-	17	-

Table.3. Minimum Bactericidal concentration of the selected plant extracts

S.NO	ORGANISMS	Minimum Bactericidal Concentration(mg/ml)			
		Punica granatum	*Psidium guajava*	*Prosopis juliflora*	*Aegle marmelos*
1.	*Staphylococcus aureus*	4	8	4	-
2.	*Klebsiella pneumonia*	8	16	4	-
3.	*Bacillus subtilis*	8	8	8	-
4.	*Salmonella typhi*	8	16	64	-
5.	*Shigella dysenteriae*	8	8	16	-
6.	*Escherichia coli*	4	16	16	-
7.	*Pseudomonas aeruginosa*	4	32	16	-
Fungi					
8.	*Candida albicans*	16	32	16	32
9.	*Mucor rouxii*	8	8	16	32
10.	*Penicillium citrenigrum*	8	8	8	32
11.	*Aspergillus niger*	32	64	32	-
12.	*Fusarium oxysporum*	16	32	32	16

Prosopis juliflora, a member of family Leguminosae, is found in arid and semi-arid regions of India. It has been used as a folk remedy for catarrh, cold, diarrhea, dysentery, excrescences, flu, hoarseness, inflammation, measles, sore throat and in healing of wounds [25]. Several alkaloids have been isolated from leaf extracts having pharmacological properties [26,27]. Apart from alkaloids, other important compounds isolated from *P.juliflora* include flavone glycoside Patulitrin, Prosogerin D, Procyanidin, ellagic acid, tannin and polystyrenes[28]. Hari Prasad *et al*., [29] had demonstrated the protective role of *Prosopis juliflora* against *Staphylococcus aureus* induced hepatotoxicity. This plant possesses antibacterial and antifungal activity comparable to that of *Punica granatum* extracts. MBC ranged from 4 to 32mg/ml.

Guava leaves contain tannins, pentacyclic, triterpenoid guajaroic acid, Uvaol, oleanolic acid, Urosolic acid, maslinic acid, Volatile oils, triterpenoids and flavonoids[30,31]. guajaverin, quercetin [32,33]. Antibacterial activity had been demonstrated by various authors [34-36, 38, 16]. Henie *et al.* [34], had reported a MIC value of 10 µg. MBC of *Psidium guajava* was found to be 8 to 64mg/ml. Chanchel and Amit kumar Das [37], had reported significant hepatoprotective activity of *Psidium guajava* leaves.

Aegle marmelos was not bactericidal at the concentration tested. In agar well diffusion method, zone of inhibition was observed at higher concentrations. Saroj Kothari *et al.*(39), had reported an MIC value of 1.25-10mg/ml with *Aegle marmelos*. According to their reports, petroleum ether extracts had comparatively higher zones of inhibition then other solvents. *Aegle marmelos* is becoming popular because of its inability to cause side effects and combat antibiotic resistant microorganisms (40).Various chemical constituents have been isolated and identified from this plant Marmelosin, Marmerin (41), Aeglin, Aegelenine, fragnine, palmitic, stearic, oleic, linolenic acids[42]) and quinolone (43]. This plant also possess antidiabetic (44), antiulcer (45), antioxidant (46), antimalarial (47), anti-inflammatory(48), anticancer (49), antiviral (50) activities and the leaf extract was found to regulate thyroid hormone (51).These leaves also were non-toxic (52).

Phytochemical analysis of the plant extracts showed the presence of alkaloids, flavonoids, saponins steroids, glycosides, phenolics and tannins. The Phytochemical analyses of different plants were shown in Table.4.

Table.4.Phytochemical analysis of the plant extracts

Sl.No.	Phytochemical	***Punica granatum***	***Psidium guajava***	***Prosopis juliflora***	***Aegle marmelos***
1.	Alkaloids	+	+	+	+
2.	Flavonoids	+	+	+	+
3.	Saponins	-	-	-	+
4.	Tannins	+	+	+	+
5.	Glycosides	+	+	+	+
6.	Phenolics	+	+	+	+
7.	Steroids	+	+	-	+

All the four medicinal plants tested, showed antibacterial and antifungal activities. Particularly *Punica granatum*, *Prosopis juliflora* and *Psidium guajava* showed very high antibacterial and antifungal activities that prove their use in traditional medicine. Extensive research is required to find out the various phytochemicals of these medicinal plants and the mechanisms of action as well as bioactivity of the various phytochemicals.

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