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Antimicrobial effects of the Flavonoid fractions of *Mimosa pudica* L. Leaves

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ABSTRACT

Mimosa pudica L. is a creeping annual or perennial herb. It has been identified as Lajjalu in Ayurveda and has been found to have antiasthmastic, aphrodisiac, analgesic and antidepressant. Compounds of pharmacological interest (flavonoids) were isolated from the plant species, *Mimosa pudica* L. and assayed against the bacteria, *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Salmonella typhi* and *Proteus mirabilis* using agar diffusion method. Flavonoids exhibited antibacterial activities against all the tested microorganisms. *P.vulgaris* was the most resistant to flavonoids isolated from the plant material followed by *S.aureus , B.subtilis, E.coli, S. typhi and P. aeruginosa*. Our findings confirm that the traditional therapeutic claims for this plant, in near future surely be able to replace the conventional antimicrobial agents to which there is increased incidence of drug interactions and the study suggests that this plant is promising for development of phytomedicine for antibacterial properties.

Key words: Flavonoid fractions, antibacterial activity, ethanol

INTRODUCTION

Plants are sources of bioactive phytocompounds and they are used as therapeutic agents, as well as starting materials for the synthesis of drugs or as models for pharmacologically active compounds. There is growing interest in the use of natural substances, generally known as bioactive phytocompounds, as antimicrobial agents (Banso and Mann, 2008). Flavonoids constitute a major group of phenolic compounds in plants. They provide pigmentation for fruits, flowers and seeds to attract pollinators and seeds dispersers. They assist in plant defense against pathogenic microorganism. The number of flavonoids is constantly increasing due to the structural variation associated with these compounds. It is well known that antioxidant activity in higher plants has often been associated with phenolic compounds (Aderogba *et al*., 2006).

Mimosa pudica L. is a creeping annual or perennial herb often grown for its curiosity value, as the compound leaves fold inward and droop when touched and reopens within minutes. It belongs to the Fabaceae family. *Mimosa pudica* is native to Brazil, but is now a pan tropical weed. The other names given to this plant are Humble plant, Shame plant, Touch me not, Sleeping grass, Prayer plant, The species epithet "pudica" is a latin equivalent for "Bashful" or "Shrinking", because of its curious nature and easy procreation. The stem is erect in young plants, but becomes creeping or trailing with age. The plant grows to a height of 1.5m (5 ft). The leaves are bipinnately compound, with one or two pinnate pairs and 10-26 leaflets per pinna. The petioles are also prickly and on close examination, it is seen that the floret petals are red in their upper part and the filaments are pink to lavender. The fruit consists of clusters of 2-8 pods of 1-2cm long each, prickly on the margins. The pods break into 2-5 segments and contain pale brown seeds 2.5mm long (Gandhiraja *et al*., 2009).

This plant has a history of use for the treatment of various ailments and the most commonly used plant part for this purpose is the root, but flowers bark and fruit can also be utilized. Several research works have been carried out to study about the phytochemical components of *Mimosa pudica* (Ahmad and Beg, 2001) and also about the antimicrobial activity of the plant (Ojalaa, *et al*., 1999). The present study intends to study about the antibacterial Activity of the crude flavonoids of *M.pudica* against selected Microbes.

MATERIAL AND METHODS

Plant Material

The botanical identity of *M.pudica* was confirmed by Dr.V.Sampath Kumar, Scientist – C, Botanical Survey of India (Southern Circle), Coimbatore, Tamilnadu. A voucher specimen has been deposited at the Department of Microbiology (MB – 004), RVS College of Arts and Science, Sulur, Coimbatore, Tamilnadu, India.

Preparation of the Polysaccharides, Proanthocyanidins and Flavonoids

The hot water extract was prepared by boiling 200 g of fresh leaves with 1 ltr. of distilled water for 3h. The final volume was reduced to 200 ml. The water extract was centrifuged and the supernatant was obtained. Excess of ethanol was added to the supernatant to precipitate the high molecular weight polysachharide fraction (800mg), which was filtered and concentrated in vacuum and extracted with ethyl acetate. The ethyl acetate soluble fraction (flavonoid fraction – 1.2g) and the aqueous fraction (Proanthocyanidin 8 g) were obtained. This resulted 12 % flavonoid fraction (Chandrika *et al*., 2006).

Determination of flavonoids

A small piece of magnesium ribbon was added to ethyl acetate extract of leaves of *M.pudica*. This was followed by drop wise addition of concentrated hydrochloric acid. Colours ranging from orange to crimson are indicative of the presence of flavonoids.

Bacterial Strains

Microorganisms were obtained from the Microbial Type Culture Collection Centre (MTCC), Chandigarh, India. Amongst seven microorganisms investigated, two Gram-positive bacteria were *Staphylococcus aureus* while five Gram-negative bacteria were *Proteus mirabilis* MTCC 425, *Escherichia coli* MTCC 2961, *Pseuodomonas aeruginosa* MTCC 4676, *Klebsiella pneumoniae* MTCC 432 and *Salmonella typhi* MTCC 733. All the microorganisms were maintained at 4^0C on nutrient agar slants.

Antimicrobial activity

Antimicrobial activity was carried out by the disc diffusion method. Sterile paper discs (6 mm in diameter) prepared from Whatman No 1 filter paper was impregnated with drug, containing solution placed on the inoculated agar. Negative and Positive controls used ethyl acetate and Chloroamphenical (Doss *et al*., 2011). The inoculated plates were incubated at 37 ^{0}C for 24 h. The antibacterial activity was evaluated by measuring the diameter of the inhibition zone for the test microorganisms. Each assay in this experiment was replicated three times.

Examination of Mode of Action

The flavonoid and proanthocyanidin were added to 4.9 ml of bacterial cultures (10^3 CFU/ml). After incubation at optimal temperature for 24 h, 100 µl of the mixtures were inoculated into 4.9 ml of fresh culturing broth. As a control, 100 µl of untreated cultures of bacteria at a concentration of 10^3 CFU/ml were transferred to 4.9 ml of fresh culturing broth. The optical density at a wave length of 600 nm (OD 600nm) of the tested and control cultures were determined at the time of inoculation and after incubation for 24h (Rattanachaikunsopon and Phumkhachorn, 2010).

RESULTS AND DISCUSSION

The presence of antibacterial substances in the higher plants is well established. Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines or it can be the base for the development of a medicine, a natural blueprint for the development of a drug (Doss *et al*., 2009).

The results showed that the flavonoids were able to inhibit all of the bacteria used in this study with different degree of inhibition. *M.pudica* leaves have long been recognized for their antibacterial activity. They were shown to inhibit both gram positive and gram negative bacteria such as *Staphylococcus aureus, Bacillus subtilis, Proteus micablis, Pseudomonas aeruginosa, Escherichia coli,* and *Salmonella typhi* (Table 1). From there results, it is possible that the flavonoids may be used as natural antimicrobial substance to replace antibiotics for controlling bacterial infections.

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Table 1. Antibacterial effects of crude flavonoids and Proanthocyanidins

Microorganisms	Mean diameter of Zone of Inhibition (mm) ± SD		
	Flavonoids	Proanthocyanidins	Chloromphenicol
Staphylococcus aureus	18.1 ± 0.15	10.0 ± 0.11	22
Bacillus subtilis	14.1 ± 0.20	8.13 ± 0.11	17
Proteus mirabilis	19.9 ± 0.15	11.16 ± 0.20	20
Pseudomonas aeruginosa	11.0 ± 0.15	8.93 ± 0.05	18
Escherichia coli	14.9 ± 0.11	8.03 ± 0.15	21
Salmonella typhi	14.1 ± 0.20	9.21 ± 0.21	16

Table 2. Recovery ability of the flavonoids and proanthocyanidin inhibited bacteria.

Microorganisms	[a]Optical Density 600_{nm}			
	Flavonoids		Proanthocyanidin	
	[b]0	[b]24	[b]0	[b]24
Staphylococcus aureus	0	0.73± 0.001	0	0.81 ± 0.15
Bacillus subtilis	0	0.70 ± 0.02	0	0.85 ± 0.11
Proteus mirabilis	0	0. 76 ± 0.01	0	0.61 ± 0.15
Pseudomonas aeruginosa	0	0.40 ± 0.01	0	0.85 ± 0.10
Escherichia coli	0	0.72 ± 0.02	0	0.89 ± 0.011
Salmonella typhi	0	0.62 ± 0.01	0	0.71 ± 0.10

[a]OD 600_{nm} (Optical Density at a wave length of 600nm). Values are the mean of three replicates.
[b]Time after inoculation of the flavonoids – Proanthocyanidin bacteria into fresh broth (h)

The antibacterial effects of the flavonoid fraction from *M.pudica* indicated that the plant has activity against all the tested bacterial strains. This observation agrees with the reports of Leven *et al*. (1979), Scherbonvaski, (1971) and Banso & Mann, (2008) both reports linked the antibacterial properties of plants to the presence of phytocompounds such as tannins, alkaloids, flavonoids and saponins. The antibacterial activity of flavonoids is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Tsuchiya *et al*., 1996).

In order to determine whether the flavonoids had bacterial or bacteriostatic mode of action on the sensitive bacteria, the abilities of the flavonoid and proanthocyanidin inhibited bacteria to resume their growth in flesh culturing broth was observed there results suggested that the flavonoids had bacteriostatic mode of action on the bacteria (Table 2). The use of antimicrobial substances with bacteriostatic mode of action may have less side effects than those with bactericidal mode of action .The latter ones tend to kill all of the bacteria in the body including normal flora whereas the former ones just retard the growth of the bacteria which are further killed by the immune response of the body .By the way, normal flora which is not normal by the immune responses is only temporary inhibited.

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