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ANTIBACTERIAL AND ANTINOCICEPTIVE ACTIVITY STUDY OF METHANOL EXTRACT OF *MIMOSA PUDICA* (L.)

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Abstract: The methanol extract of *Mimosa pudica* was studied for its antibacterial activity against twelve pathogenic bacteria. The extract was also studied for its effect on acetic acid induced writhing model in mice. The extract showed good activity against two gram-positive and six gram-negative bacteria but failed to show any activity against *Staphylococcus pyogenes*, *Salmonella typhi, Shigella boydii* and *Vibrio cholera*. The extract exhibited no antinociceptive activity in acetic acid induced writhing test; rather it increased the abdominal constriction or writhing as compared to the control group which is a sign of pain sensation.

Key words: *Mimosa pudica*, antibacterial activity, antinociceptive activity

Introduction

M. pudica. (Fam: Mimosaceae), locally known as lajjaboti (Eng. Sensitive plant) is a stout, strangling, shrubby plant with leaves sensitive to touch, spinous stipules and globose pinkish flower heads is found to grow wild as weed in almost all parts of Bangladesh, India, Pakistan, Bhutan, Srilanka and in other parts of Asia. The plant is used in the treatment of diarrhoea, dysentery, leucorrhoea and in morbid condition of vagina (Kiritikar and Basu, 1999). The plant is also useful in sore gums, as a blood purifier and in treating convulsions of children (Ghani, 1998). The leaves and roots are used in piles, fistula and in vaginal and uterine complaints (Yusuf *et al.*, 1994). The leaves are reported to possess hyperglycemic effect (Amalraj and Ignacimuthu, 2002). The objective of the present work was to investigate whether the methanolic extract of the plant *M. pudica* possesses any antibacterial and antinociceptive activity.

Materials and Methods

Plant material and extraction: The plant *M. pudica* Linn. was collected from the campus of Khulna University, Khulna in January 2003 and was identified by the experts of Bangladesh National Herbarium. The dried whole plant was pulverized into coarse powder (350 g) and macerated with methanol (1200 ml) at room temperature for overnight. The extract was filtered and the solvent was evaporated off under reduced pressure in a rotary evaporator (approx. yield 2 %). Amount of the extract obtained was 7.21 g.

Animals: Swiss albino mice of either sex (22-25 g) obtained from the Animal Resources Branch of the International Center for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) were used. The animals were housed under standard environmental condition and fed with standard diet (ICDDR, B formulated) and water *ad libitum*.

Antibacterial activity study: The sensitivity of methanol extract of *M. pudica* against a number of pathogenic microorganisms was investigated *in vitro* by agar well diffusion method (Russel and Furr, 1997;

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Cruickshank, 1968). With the help of a sterile loop, a small amount of bacterial culture from the supplied nutrient agar culture slant was inoculated into 100 ml of sterilized nutrient broth in 250 ml conical flask and kept at 37 °C in a slow shaking incubator for overnight. After 24 hrs of growth, the broth of bacteria was ready for use as inoculums for antibacterial sensitivity test.

Cork borer (diameter: 6 mm) was sterilized in an autoclave at 160 °C for 15min. The crude extract was dissolved in methanol to get the concentration of 50 mg ml^{-1}. Standard antibiotic solution (Gentamycin 5 mg ml^{-1} in sterile water) was used as control. Sterilized nutrient agar (20 ml) (40-50 °C) was poured into each Petri dish containing 0.1 ml of broth culture of bacteria (inoculums). Then the Petri dish was kept in 0^oC temperature for 1hr. With the help of cork borer the bores were produced on the Petri dish by scarping the media. Sample solution and standard antibiotic solution were given in the bore on the seeded agar plates with the help of micropipette. All the plates were kept in a refrigerator for 5-6 hrs for complete diffusion. They were then incubated at 37 °C for 24 hrs. All the determinations were carried out in triplicate and average zone of inhibition was recorded.

Effect on acetic acid-induced writhing: Effect on nociception was studied using acetic acid induced writhing model in mice (Whittle, 1964). After an overnight fast, the animals were divided into control, positive control and test groups containing five mice in each group. The animals were fed with test substance at the doses of 250 and 500 mg kg^{-1} body weight, reference drug (aspirin) and control vehicle 45 min before intraperitoneal administration of 0.7 % acetic acid. After a five minutes interval for proper absorption of acetic acid, the mice were observed for specific contraction of body referred as 'writhing', which is an indication of pain sensation in test animals. A comparison of writhing was made between positive control, control and test sample.

Results

The *in vitro* antibacterial activity study was designed to investigate the antibacterial spectrum of the methanol extract of *M. pudica*. Results of this study indicated that the extract has moderate antibacterial activity against a number of pathogenic gram-positive and gram-negative bacteria tested (Table 1).

In the acetic acid induced writhing test, the standard drug, aspirin used in this test significantly reduced the writhing in test animals. But the extract did not show writhing inhibition to any degree, rather it increased the writhing as compared to the control group (Table 2). Though the result for the dose level of 250 mg kg^{-1} was not statistically significant, but the result for 500 mg kg^{-1} dose level had a high statistical significance as compared to the control. Since acetic acid induced writhing model represents pain sensation by peripheral mechanism of prostaglandin synthesis, the result of present work indicated that *M. pudica* extract might have agent(s) that induce pain by prostaglandin synthesis.

Table 1. *In vitro* antibacterial activity of methanol extract of *M. pudica*.

Microorganisms		Zone of inhibition (mm)	
		Methanol extract (50 mg/ml)	Gentamycin (5 mg/ml)
Gram-positive	*Staphylococcus aureus*	12	30
	Staphylococcus epidermis	16	26
	Staphylococcus pyogenes	0	30
Gram-negative	*Enterobacter aerogenes*	18	32
	Escherichia coli	15	28
	Pseudomonas aeruginosa	16	23
	Salmonella typhi	0	29
	Shigella boydii	0	32
	Shigella dysenteriae	19	30
	Sigella flexneri	17	30
	Shigella sonnei	18	33
	Vibrio cholera	0	32

Table 2. Effect of methanol extract of *M. pudica* on acetic acid induced writhing in mice.

Treatment	Dose[a] (mg kg^{-1}, p.o.)	Writhings[b]	% Writhing
Control (1% Tween 80, 10 ml kg^{-1}, p.o.)	-	32.6 ±1.89	100
Aspirin	50	6.4 ±0.75*	19.63
M. pudica	250	37±1.64	113.50
	500	46.2±1.66*	141.72

[a]Administered 45 min before 0.7% acetic acid administration (ml kg^{-1}, i.p.);
[b]Counted for 15 min, starting 5 min after acetic acid administration; values are mean ±S.E.;
*p<0.001 vs. control, Student's *t*-test; n=5.

Discussion

The gram-positive bacteria include *Staphylococcus aureus, Staphylococcus epidermis* and the gram-negative bacteria include *Enterobacter aerogenes*, *Escherichia coli, Pseudomonas aeruginosa, Shigella dysenteriae, Sigella flexneri, Shigella sonnei.* But the extract failed to inhibit the growth of *Staphylococcus pyogenes* (gram-positive), *Salmonella typhi, Shigella boydii* and *Vibrio cholera* (gram-negative). Traditionally the plant has been used to treat various infectious disease (Kiritikar and Basu, 1999) and the result from this current study revealed the scientific basis of the traditional usage of *M. pudica.*

Intraperitoneal administration of acetic acid causes algesia by liberating noxious endogenous substances including serotonin, histamine, prostaglandin, bradykinin and substance P that sensitize pain nerve endings (Collier *et al.*, 1968; Raj, 1996). Of the prostanoids, mainly prostacycline (PGI_2) has been held responsible for the causation of pain following acetic acid administration (Murata *et al*., 1997). It has been suggested that acetic acid stimulates the vanilloid receptor and bradykinin B_2 receptor in the pathway comprising sensory afferent C-fibers (Ikeda *et al.*, 2001). The reason behind the observed activity of the methanolic extract of *M. pudica*, may be due to the effect of the extract in increasing the synthesis and/or release of those endogenous substances or an excitatory effect of the extract on the nerve fibers involved in the pain transmission pathway.

Conclusion

The antibacterial activity of methanol extract of *M. pudica* against a number of gram-positive and gram-negative bacteria supports the rationale for its ethno pharmacological uses in the treatment of diseases caused by bacteria. Since *M. pudica* extract inhibited the growth of *Staphylococcus aureus, Staphylococcus epidermis,* bacteria responsible for skin infections, the extract could be used externally in the treatment of superficial infections caused by above microorganisms. As *M. pudica* extract showed an increased pain sensation in test animals, its oral administration should be further investigated to evade any untoward effect. Further studies could be carried out to establish this preliminary finding and to isolate the active principle (s) responsible for such activities.

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