

Phytochemical and Pharmacological Screening of Combined *Mimosa pudica* Linn and *Tridax procumbens* for *In vitro* Antimicrobial Activity

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Abstract: Treatment of infections continues to be problematic in modern time because of the severe side effects of some drugs and the growing resistance to antimicrobial agents. An extract of the leaves of *Mimosa pudica* Linn possesses aphrodisiac, antipyretic, antispasmodic, anticancer and diuretic actions. Six crude extracts were prepared from the whole plant *Mimosa pudica* Linn using different solvents by cold maceration process. *Tridax procumbens* Linn is a tropically distributed medicinal plant. Antimicrobial activity of aqueous extracts of two plants were investigated by agar disc well-diffusion method against bacterial pathogens *Staphylococcus aureus*, *Bacillus subtilus*, *Escherichia Coli*, *K. Pneumonia*, *M. luteus* and *C. Albicans* and were compared to Ciprofloxacin, Gentamycin and Gatifloxcin as standard. The plant extracts showed inhibitory activity against the tested organisms. Phytochemical screening of the plant revealed the presence of tannins, flavonoids, saponins and alkaloids. In conclusion, this study scientifically validated the use of plant in traditional and ethnoveterinary medicine.

Key words: Antibacterial • Antifungal • *Mimosa pudica* Linn • *Tridax procumbens*

INTRODUCTION

Many efforts have been done to discover new antimicrobial compounds from various kinds of sources such as soil, micro organisms, animals and plants. One of such resources is folk medicine and systematic screening of them may result in the discovery of novel effective compounds [1]. There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world [2-4]. Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, recently much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine [5]. During last ten years the phase of development of new antimicrobial drugs has slowed down while the prevalence of resistance bacteria is no longer matched by expansion in the arsenal of agents [6]. *Mimosa pudica* has been extensively used in ayurveda, unani & homoeopathic medicine and has become cynosure of modern medicine. It is known in sensitive or humble plant & popular name is lajjavanti and chuimui [7]. *Mimosa pudica* posses a wide area of therapeutic activity likes vulnerary, diuretic, antispasmodic, emetic, constipating and febrifuge.

They used in haemorrhage, dysentery, inflammation, burning sensation & wounds. It is also used in jaundice, asthma, conjunctivitis, cut wounds and glandular swelling [8-9]. *Tridax procumbens* Linn (compositae) is a common grass found in tropical areas of all countries, growing primarily during rainy season. It is a common weed in Madhya Pradesh India, present along with economically important crops. It habitats waste places, road sides and hedges throughout India. It is denoted by different names; in English as Mexican Daisy, in Ayurvedic as Jayanti, in Siddha/Tamil as Vettukkaaya-thalai and in Folk as Akala Kohadi. The exomorphology and histomorphology of leaf, petiole, internode and root of this plant were studied [10]. The extracts of *T.procumbens* have been reported to have various pharmacological effects including antimicrobial activity, wound healing property and immunomodulatory activity on the experimental animals [11-13].

The main aim of the present investigation was to study the antimicrobial activity and preliminary phytochemical screening of combined *Mimosa pudica* Linn and *Tridax procumbens* extract in different solvent like hexane, chloroform, ethyl acetate, methanol, ethanol and water.

MATERIALS AND METHODS

Collection and Identification of Plant Material:

The whole plant was collected from the wet lands of Indore in the month of September. The plant was identified and confirmed by a pharmacologists; a voucher specimen was deposited at the herbarium in the institute.

Preparation of Plant Extracts: The whole plant including the flower heads was shade dried and coarsely powdered with electric blender. The powdered drug passed through sieve number 40 to obtain uniform powder and packed in airtight sealed envelopes for further studies. Then it was extracted by maceration at room temperature for 7 days with regular stirring after every 2 hrs in the order of increasing polarity with hexane, chloroform, ethyl acetate, methanol, ethanol and water. The extracts were collected and concentrated at 40°C under reduced pressure using rotary evaporator. The extract was stored at 4°C until further use for various evaluations. These extracts were used to conduct the phytochemical and pharmacological evaluation of *Mimosa pudica* Linn and *Tridax procumbens*.

Phytochemical Screening Test: Phytochemical screening is done for analyzing secondary metabolites, which are responsible for curing ailment. The phytochemical screening of the plant extract was carried out by the methods used [14-17] to detect the presence or absence of certain bioactive compounds.

Antimicrobial Activity

Culture Media and Inoculums: Muller Hinton broth (MH) and Nutrient broth (NB) media (Hi-Media Pvt. Ltd., Bombay, India) were used for NCIM. The cultures used were incubated at 37°C for 24 hrs.

Preparation of Bacteria: The bacteria *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia Coli*, *K. Pneumonia*, *M. luteus* and *C. albicans* were purchased from DAVV Indore, India. The ability of the various extracts to inhibit growth of clinical bacteria and fungi isolates was determined using the Agar disc diffusion method. The microorganisms were inoculated into SBCB and incubated at 35±2°C for 4 hrs. The turbidity of the resulting suspension was diluted with SBCB to match with 1.5 McFarland turbidity standard. This level of turbidity is equivalent to approximately 4.0 × 10⁶ CFU/ml.

Agar Well Diffusion Method: The modified agar well diffusion method was employed [18-20]. Muller-Hinton

agar plates were inoculated by streaking the swab over the entire sterile over the entire sterile agar surface. The agar well diffusion method was carried out to evaluate antibacterial activity. Test organism was spread on Muller-Hinton agar plates. The standard inoculums (NCIM cultures) were evenly spread on the surface of the medium then wells of 6mm diameter were punched into the agar medium and filled with 60 µl (5mg/ml) of *Mimosa pudica* plant extract of various concentration (150-450 µg) were dissolved in DMSO. Six wells were made, in each well different concentration of extract is added and 10 µl of Ciprofloxacin (which is used as standard) was filled in and this plate is kept in refrigerator for 20 minutes for diffusion. The plates were incubated for 24 hours at 37°C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism.

Thin Layer Chromatography: Successive extractions with hexane, ethyl acetate and methanol were carried out on the pulverized sample by maceration for 24 hrs. The extracts were filtered and concentrated on a rotary evaporator. Each concentrated extract was spotted on a normal phase plate previously activated at 110°C for 2 hrs, using a capillary tube. The plate was developed using mobile phase of hexane-ethyl acetate (12:3) for hexane extract and hexane-ethyl acetate (7:3) for ethyl acetate and methanol extracts.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of ethanolic extract of *Mimosa pudica* Linn and *Tridax procumbens* revealed the presence of alkaloids, flavonoids, saponins, terpenoids, tannins and Phenolics (Table 1). Most of the secondary metabolites were identified in the polar extracts. The concentration of polar metabolites is higher than non-polar metabolites in leaves of these species.

Table 1: Phytochemical group test for the *Mimosa pudica* Linn and *Tridax procumbens*

S. No.	Phytoconstituents	Extract of leaves of <i>Mimosa pudica</i> Linn
1	Alkaloid	+
2	Amino acid	-
3	Flavonoids	-
4	Glycosides	-
5	Tannins	+
6	Saponins	+
7	Phenolics	+
8	Flavonoids	+

+Present, - Absent

Table 2: Antimicrobial activity of *Mimosa pudica* Linn and *Tridax procumbens* aqueous extract of different micro organisms

Sample Conc. in %	Zone of Inhibition in (mm)					
	<i>S. Aureus</i>	<i>B. Subtilis</i>	<i>E. Coli</i>	<i>K. pneumoniae</i>	<i>M. luteus</i>	<i>C. albicans</i>
150	26	19	28	22	27	22
250	24	16	23	20	24	22
350	20	18	24	18	20	19
450	25	20	18	17	19	20

Table 3: Antimicrobial activity of antibiotics on different micro organisms *Mimosa pudica* Linn and *Tridax procumbens*

Standard Drug	Microorganisms (inhibition zone in mm)					
	Gram-positive			Gram-negative		
	<i>S. aureus</i>	<i>B.subtilis</i>	<i>K. pneumoniae</i>	<i>E.coli</i>	<i>M. luteus</i>	<i>C. albicans</i>
Ciprofloxacin	29	24	16	18	23	15
Gentamycin	26	22	-	-	19	13
Gatifloxacin	23	25	18	16	12	17

Alkaloids are one of the characteristic secondary metabolites in leaves of this genus. Flavonoids are known to be synthesized by plants in response to microbial infection. Tannins (commonly referred to as tannic acid) are also known as antimicrobial agents. They are water-soluble polyphenols and precipitated proteins present in many plant foods. Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein. Now a day the standardization of crude drugs has become very important for identification and authentication of drug. But, due to certain problems the importance was not up to the mark. Thus, the lack of standardization technique fails to identify the drug from its originality which there by exploits the usage of drug from its Traditional System of Medicine. The antibacterial activity is measured by zone of inhibition (mm). Total six bacterial strains were used in this investigation. The observed antimicrobial activity against the tested organisms could be due to the presence of tannins and cyanogenetic glycosides in the extract as these have previously been reported to possess antimicrobial activities. These could explain the rationale for the use of the plant in the treatment of the various conditions in traditional medical practice. The results seem to justify their continued use in the treatment of microbial infections. The inhibition of growth of the test organisms that are known to cause nosocomial infections and displaying multidrug resistance to most antibiotics and non-antibiotic antimicrobial agents justify the continued use of these plants in folk and traditional medical practice. Further investigation on the isolation and identification of anti microbial component(s) in the plant may lead to

chemical entities with potential for clinical use. The antimicrobial affect of plant extract against the different strains are illustrated in Table 2. The extract of *Mimosa pudica* Linn and *Tridax procumbens* at the concentration of 100% has antimicrobial activity on the tested microorganism form high to low respectively. *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia Coli*, showed in (Table 2). The data indicated that Gram negative was the most sensitive strain of those tested with the extract of *Mimosa pudica* Linn with strongest inhibition zone of 28 mm. The extract concentration of 100 % also exhibit high antimicrobial activity against *Staphylococcus aureus*, with modest activity against, *Bacillus subtilis*, *K. pneumoniae*, *Escherichia Coli*, *C. albicans*. The 75 % concentration of the extract of *Mimosa pudica* Linn also showed strongest inhibition zone against different strains of microorganisms. The data indicated that anti-microbial activity of extract (at 50 % concentration) with strongest inhibition zone of 22 mm.

In conclusion further pharmacological and clinical studies are required to understand the mechanism and the actual efficacy of these herbal extracts in treating various infections and skin diseases like psoriasis. Aqueous extracts of rhizomes of *Mimosa pudica* Linn and *Tridax procumbens* exhibited better antibacterial activity as compared to their petroleum ether, methanolic and ethanolic extracts.

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REFERENCES

1. Janovska, D., K. Kubikova and L. Kokoska, 2003. Screening for antimicrobial activity of some Medicinal plant species of traditional Chinese medicine. Czech J. Food Sci., 21: 107-111.
2. Chung, P.Y., L.Y. Chung and Y.F. Ngeow, 2004. Antimicrobial activities of Malaysian plant species. Pharm Biol., 42: 292-300.
3. Nair, R., T. Kalariya and S.V. Chandra, 2004. Antibacterial activity of some medicinal plants of Saurashtra Region. J. Tissue Res., 4: 117-120.
4. De Boer, H.J., A. Kool and A. Broberg, 2005. Antifungal and antibacterial activity of some herbal remedies from Tanzania. J. Ethnopharmacol., 96: 461-469.
5. Essawi, T. and M. Srour, 2005. Screening of some Palestinian medicinal plants for antibacterial activity. J. Ethnopharmacol., 70: 343-349.
6. Yuldasheva, L.N., E.B. Carvalho, M.T.J.A. Catanho and O.V. Krasilnikov, 2005. Cholesterol dependent haemolytic activity of *Passiflora quadrangularis* leaves. Ed. 7th 38: 1061-70.
7. Akinpelu, D.A. and T.M. Onakoya, 2006. Antimicrobial activities of medicinal plants used in Folklore remedies in south-western. African J. Biotechnol., 5(11): 1078-1081.
8. Sharma, P.C., M.B. Yelne and T.J. Dennis, 2005. Database on Medicinal Plants Used In Ayurveda. Vol.3. New Delhi; Central Council for Research in Ayurveda and Siddha, pp: 282-284.
9. John, A.P., 2001. Healing Plants of Peninsular India. USA; CABI publishing, pp: 557-563.
10. Suseela, L., A. Sarsvathy and P. Brindha, 2002. Pharmacognostic studies on *Tridax procumbens* L. (Asteraceae). J. Phytological Res., 15: 141-147.
11. Diwan, P.V., L.D. Tilloo and D.R. Kulkarni, 1982. Influence of *Tridax procumbens* on wound healing. Indian J. Med. Res., 75: 450-454.
12. Diwan, P.V., L.D. Tilloo and D.R. Kulkarni, 1983. Steroid depressed wound healing and *Tridax procumbens*. Indian J. Physiol. Pharmacol., 27: 32-36.
13. Udopa, S.L., A.L. Udopa and D.R. Lalkarni, 1991. Influence of *Tridax procumbens* on lysyl oxidase activity and wound healing. Planta Med., 57: 325-7.
14. Bhattacharjee, S.K., 1989. Handbook of Medicinal Plants. Edn. 1st Calcutta, Medical Allied Agency, pp: 3-4.
15. Gibbs, R.D., 1974. Chemotaxonomy of flowering plants, Vol. 1, McGill-Queens University Press- N, Montreal- London, pp: 455-459.
16. Trease, G.S. and H.C. Evans, 1978. Textbook of Pharmacognosy. Bailiar Zindall and Co. London Publishers, pp: 876-881.
17. Harborne, J.B., 1984. Phytochemical methods, Chapman and Hall, London Publishers, pp: 353-355.
18. Peach, K. and M.V. Tracey, 1955. Modern Methdender Pflanzen analyse, Vol.III. Springer Verlag, Berlin, pp: 626.
19. Spooner, D.F. and G. Sykes, 1972. Methods in Microbiology, Vol. VII B, Academic Press, London and New York, pp: 216.
20. Nair, R. and S. Chanda, 2005. Antibacterial activity of *Punica granatum* in different solvents. Indian J. Pharmaceutical Sci., 67: 239-243.