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PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND ANTIPYRETIC ACTIVITY OF *MIMOSA PUDICA*

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Abstract:

Plants either in raw form or their isolated bioactive constituents are utilized as complementary and alternative medicine in various disorders. The present study was undertaken to evaluate in vitro antioxidant and antipyretic activities of the Hydroalcoholic extract of the leaves of Mimosa pudica. DPPH scavenging activity was measured by the spectrophotometer for ascorbic acid and hydroalcoholic extract of Mimosa pudica. The yeast-induced hyperthermia in the rat model was, therefore, employed to further investigate the antipyretic activity of mimosa pudica. Extract was administered i.p. 30 min before the injection of the pyrogen. The rectal temperature of animals was recorded at 1 h intervals for 4 h following the administration of drug or plant extract. This result seems to support the view that the plant has some influence on prostaglandin biosynthesis, since prostaglandin is believed to be a regulator of body temperature.

Key words: *Mimosa pudica, Hydroalcoholic, Antioxidant and Antipyretic activity*

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INTRODUCTION:

Pyrexia or fever is caused as a secondary impact of infection, malignancy or other diseased states. It is the body's natural defense to create an environment where infectious agent or damaged tissue cannot survive [1]. Normally the infected or damaged tissue initiates the enhanced formation of proinflammatory mediator's (Cytokines like interleukin 1β, α, β and TNF- α), which increase the synthesis of prostaglandin E2 (PG E2) near peptic hypothalamus area and thereby triggering the hypothalamus to elevate the body temperature [2]. As the temperature regulatory system is governed by a nervous feedback mechanism, so when body temperature becomes very high, it dilate the blood vessels and increasing sweating to reduce the temperature; but when the body temperature become very low hypothalamus protect the internal temperature by vasoconstriction. High fever often increases faster disease progression by increasing tissue catabolism, dehydration and existing complaints, as found in HIV [3]. Drugs having anti-inflammatory activity generally possess antipyretic activity (e.g) non-steroidal anti-inflammatory drugs (NSAIDs). It has been suggested that prostaglandin (PGE) mediates pyrogen fever; the ability of NSAIDs, to inhibit prostaglandin synthesis could help to explain their antipyretic activity.

Fever is one of the most common presenting signs of illness in office-based primary care pediatric practice, accounting for 19% to 30% of visits [4-5]. Infants and young children are particularly susceptible to fever because of their small body size, high ratio of body surface area to weight, and low amount of subcutaneous fat. Although most experts consider fever a beneficial physiologic response to the infectious process, it can lead to patient irritability and stress as well as high parental anxiety [6]. Therefore, physicians usually prefer to prescribe antipyretic agents in addition to nonpharmacologic, physical fever-reducing modalities [7].

Ayurvedic medicines mainly based on plants enjoy a respective position today, especially in the developing countries, where modern health services are limited. Safe effective and inexpensive indigenous remedies are gaining popularity among the people of both urban and rural areas especially in India and China. Information from ethnic groups or indigenous traditional medicines has played vital role in the discovery of novel products from plants as chemotherapeutic agents. Herbal medicines have been main source of primary healthcare in all over the world. From ancient times, plants have been catering as rich source of effective and safe medicines. About 80 % of world populations are still dependent on traditional medicines.

Mimosa pudica L. is a creeping annual or perennial herb. It has been identified as lajjalu in Ayurveda and has been found to have antiasthmatic, aphrodisiac, analgesic, and antidepressant properties. *M. pudica* is known to possess sedative, emetic, and tonic properties, and has been used traditionally in the treatment of various ailments including alopecia, diarrhea, dysentery, insomnia, tumor, and various urogenital infections. Phytochemical studies on *M. pudica* have revealed the presence of alkaloids, non-protein amino acid (mimosine), flavonoids C-glycosides, sterols, terpenoids, tannins, and fatty acids.

Two well-known movements are observed in *M. pudica* L. (ojigi-so in Japanese): one is the very rapid movement of the leaves when it is stimulated by touch, heating, etc., and the other is the very slow, periodical movement of the leaves called nyctinastic movement which is controlled by a biological clock.
Fever is managed using synthetic drugs such as aspirin, paracetamol among others. Synthetic drugs are associated with many side effects. Herbal medicines form alternative therapy since they possess fewer side effects and are readily available. This study aimed to determine antipyretic potential of hydroalcoholic extract of *Mimosa pudica* L in albino rats.

MATERIAL AND METHODS:

Chemical and Reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals and solvent used in this study were of analytical grade. The pathogenic microbes used in the current study were obtained from Microbial Culture collection, National Centre Forcell Science, Pune, Maharashtra, India.

Collection of plant material

The plants have been selected on the basis of its availability and folk use of the plant. Leaves of *Mimosa pudica* was collected from local area of Bhopal in the month of February, 2022.

Extraction procedure

Following procedure was adopted for the preparation of extract from the shade dried and powdered herbs [8, 9].

Defatting of plant material

75.80 gm of dried powdered of *Mimosa pudica* leaves was coarsely powdered and subjected to extraction with petroleum ether by soxhlet extraction. The extraction was continued till the defatting of the material had taken place.

Extraction by soxhlet extraction

Defatted dried powdered leaves of *Mimosa pudica* has been extracted with hydroalcoholic solvent (ethanol : water; 80:20 v/v) using soxhlet extraction process for 24-48 hrs, filtered and dried using vacuum evaporator at 40°C.

Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

Phytochemical Screening

Phytochemical examinations were carried out for all the extracts as per the standard methods.

1. Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins

Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. Detection of phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of tannins

Gelatin Test: To the extract 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

7. Detection of flavonoids

Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

8. Detection of proteins

Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

9. Detection of diterpenes

Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Quantitative estimation of bioactive compound

Estimation of total phenolic content

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method [10].

Preparation of Standard: 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5-25μg/ml was prepared in methanol.

Preparation of Extract: 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this solution was used for the estimation of phenol.

Procedure: 2 ml of each extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method [10].

Preparation of standard: 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol.

Preparation of extract: 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this solution was used for the estimation of flavonoid.

Procedure: 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

Antioxidant activity of extract using DPPH method

DPPH scavenging activity was measured by the spectrophotometer. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance [11]. Decrease in the absorbance in presence of sample extract at different concentration (10- 100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally the mean was taken. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm.

$$\text{Calculation of } \%\ \text{Reduction} = \frac{\text{Control Absorbance} - \text{Test absorbance}}{\text{Control Absorbance}} \times 100$$

In vivo* antipyretic activity of *Mimosa pudica

Animals

Laboratory Swiss albino rats (180–200 g) of either sex were housed at 25° ± 5 °C in a well-ventilated animal house under 12/12 h light/dark cycle. The animals had free access to standard food pellets (Bird House Bhopal, India) containing (% w/w) protein 22.10, oil 4.13, fiber 3.15, ash 5.15, sand (silica) 1.12, and water ad libitum. Bedding material was removed and replaced with fresh paddy husk as often as necessary to keep the animals clean and dry. The animals were maintained under standard conditions in an animal house approved by Committee for the purpose of control and supervision on experiments on animals (CPCSEA). The experimental protocol was approved by Institutional ethical committee. The animals were subjected for quarantine (10 days) prior to experimentation.

Acute toxicity study (LD_{50}).

Acute oral toxicity test was carried out according to the Organization for Economic Co-operation and Development (OECD) guidelines for Testing of Chemicals number 420 (OECD, 2001). The study was initiated with a sighting study aimed to determine the dose for the acute toxicity study. The sighting study comprised of female Swiss albino rats dosed in a step wise procedure using the fixed doses of 5, 50, 300 and 2000 mg/kg.

Starting with 5 mg/kg BW, the test article was administered orally to one rat. The rat was then observed for toxic effect for the first 30 min followed by hourly for 8 h for the first 24 h. If they are no signs of toxic effect or mortality observed on the rat within the 24 hours, we then dosed another rat with the next dose (50 mg/kg BW) and a similar procedure was carried out. A stepwise procedure was carried out until the highest dose, 2000 mg/kg BW is reached. If all the rats survived, they were monitored and observed once daily for the next 13 days. The sighting study showed that the rats dosed with 5, 50 300 and 2000 mg/kg BW with the test article survived.

Based on this observation we decided to use the highest dose, 2000 mg/kg BW for the main test, the acute toxicity study. The acute toxicity study comprised of two groups, one control and one treatment group that consisted of 5 female rats in each group. Female rats were chosen because it is the most sensitive gender to see the effect of treatment [12].

The treatment group received *Mimosa pudica* extract that was diluted in water at a dose 2000 mg/kg BW given orally once, in a 2 ml volume. The control group received water delivered in the same volume and same procedure as the treatment group. The experimental animals were observed for 30 min after treatment, followed by observation hourly for 8 h and once daily for the next 13 days.

Brewer's yeast-induced pyrexia in rats

The antipyretic properties of *Mimosa pudica* were tested in rats in which hyperthermia had been

induced following the method of Teotino *et al.*, (1963) [12]. The initial rectal temperatures of the rats were recorded using a six channel electric thermometer connected with probes. Rats were made hyperthermic by a subcutaneous injection of 20% yeast suspension in 0.9% saline at a dose of 1 mL/100 g body weight. When the temperature was at a peak (18 h after yeast injection) the rectal temperature was recorded again. Those animals that showed a rise in rectal temperature of more than 1.2 °C were used. Test substances and control vehicle were given intraperitoneal and the rectal temperature of the animals was recorded at 1 h intervals for 4 h following the administration of drug or plant extract.

Statistical analysis

The experimental results are represented as mean ± SE (standard error of the mean). Student's t-test was used for the evaluation of data and $p < 0.05$ accepted as significant.

RESULTS AND DISCUSSION:

A small portion of the dried extracts were subjected to the phytochemical test using standard methods to test for alkaloids, glycosides, saponins, flavonoids and phenol separately for extracts of all samples. Small amount of each extract is suitably resuspended into the sterile distilled water to make the concentration of 1 mg per ml. The outcomes of the results are discussed separately in the table 2. The content of total phenolic compounds (TPC) content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: $Y = 0.042X-0.002$, $R^2= 0.999$, where X is the gallic acid equivalent (GAE) and Y is the absorbance. The content of total flavonoid compounds (TFC) content was expressed as mg/100mg of quercetin equivalent of dry extract sample using the equation obtained from the calibration curve: $Y = 0.06X+0.019$, $R^2= 0.999$, where X is the quercetin equivalent (QE) and Y is the absorbance table 3. Clinically available antipyretic drugs, such as paracetamol and the non-steroidal anti-inflammatory drugs are able to lower the body temperature only in feverish patients. Neuroleptic drugs and other central depressants can also reduce the normal body temperature.

In general, non-steroidal anti-inflammatory drugs produce their antipyretic action through inhibition of prostaglandin synthetase within the hypothalamus. Therefore, it appears that the antipyretic action of the extract may also be related to the inhibition of prostaglandin synthesis.

The yeast-induced hyperthermia in the rat model was, therefore, employed to further investigate the antipyretic activity of *mimosa pudica*. Extract was administered i.p. 30 min before the injection of the pyrogen. The rectal temperature of animals was recorded at 1 h intervals for 4 h following the administration of drug or plant extract.

Table 1: % Yield of leaves of *Mimosa pudica*

S. No.	Extract	% Yield (w/w)
1.	Hydroalcoholic	7.58%

Table 2: Phytochemical screening of extract *of Mimosa pudica*

S. No.	Constituents	Hydroalcoholic extract	Observation
1.	**Alkaloids**		
	Mayer's Test:	-ve	Green coloured
	Wagner's Test:	-ve	Green coloured
	Dragendroff's Test:	-ve	Light Green coloured
	Hager's Test:	+ve	Yellow coloured precipitate.
2.	**Glycosides**		
	Legal's test	-ve	Green coloured
3.	**Flavonoids**		
	Lead acetate	+ve	Yellow coloured precipitate
	Alkaline Reagent Test:	+ve	colourless
4.	**Phenol**		
	Ferric Chloride Test	+ve	Black coloured
5.	**Proteins**		
	Xanthoproteic test	+ve	Yellow coloured
6.	**Carbohydrates**		
	Molisch's Test:	-ve	Yellow coloured

	Benedict's Test: Fehling's Test:	-ve +ve	Yellow coloured Red precipitate
7.	**Saponins** Froth Test: Foam Test:	 +ve -ve	 Layer of foam No foam
8.	**Diterpins** Copper acetate test	 -ve	 Green coloured
9.	**Tannins** Gelatin Test:	 -ve	 Green coloured

Table 3: Total phenolic and total flavonoid content of *Mimosa pudica*

S. No.	Total Phenol content	Total flavonoid content
1.	0.463 mg/100mg	0.574 mg/100mg

Table 4: % Inhibition of ascorbic acid and extract of *Mimosa pudica* using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	*Mimosa pudica* extract
1	10	38.21	7.51
2	20	54.74	16.84
3	40	62.35	22.75
4	60	70.84	30.21
5	80	82.57	42.58
6	100	87.66	51.01
IC 50		**20.332**	**98.268**

Table 5: Effect Hydroalcoholic extract of *mimosa pudica* on Brewer's yeast-induced pyrexia in rats

Temperature	Treatment after pyrexia (°C)	Rectal temperature after administration of drug (°C)				
		0 h	1 h	2 h	3 h	4 h
Saline (2 mL/kg, i.p.)	37.53 ± 0.34	37.49 ± 0.14	37.7 ± 0.14	37.4 ± 0.14	37.36 ± 0.19	37.42 ± 0.31
Paracetamol (150 mg/kg, i.p.)	37.58 ± 0.27	37.56 ± 0.3	36.49 ± 0.12c	36.04 ± 0.64b	35.92 ± 0.18c	36.04 ± 0.41c
Extract (200 mg/kg, i.p.)	37.65 ± 0.164	37.63 ± 0.157	36.93 ± 0.08	37.04 ± 0.62	37.08 ± 0.27a	36.98 ± 0.19a
Extract (400 mg/kg, i.p.)	37.64 ± 0.133	37.58 ± 0.125	37.09 ± 0.71	37.06 ± 0.58	36.82 ± 0.13c	36.64 ± 0.68a

Extract was administered i.p. 30 min before the injection of the pyrogen. Values are expressed as mean ± SE from the experiments. The rectal temperature of animals was recorded at 1 h intervals for 4 h following the administration of drug or plant extract. a $p < 0.05$, b $p < 0.01$, c $p < 0.001$ when compared with saline-treated group.

CONCLUSION:

It was found that dose of 200 and 400 mg/kg of *mimosa pudica* showed a significant decrease in rectal temperature (36.98 ± 0.19 and36.64 ± 0.68) similar to paracetamol (36.04 ± 0.41) after 4 h interval. This result seems to support the view that the plant has some influence on prostaglandin biosynthesis, since prostaglandin is believed to be a regulator of body temperature.

REFERENCES:

1. Chattopadhyay D, Arunachalam G, Ghosh L, Rajendran AB, Bhattacharya SK.Antipyretic activity of Alstonia macrophylla Wall exA. DC: An ethnomedicine of Andaman Islands. Journal of Pharmacy and Pharmaceutical Science. 2005; 8:558-564.
2. Spacer CB, Breder CD. The neurologic basic of fever. New England Journal of Medicine. 1994; 330:1880-1886.
3. Veugelers PJ, Kaldor JM, Strathdee SA, Page-Shafer KA, Schechter MT, Coutinho RA, Keet, IP Van Grienseven GJ. Incidence and prognostic significance of symptomatic primary human immuno deficiency virus type Infection in homosexual men. Journal of Infectious Disease. 1997; 176:112-117.
4. Eskerud JRLaerum EFagerthun HLunde PK Naess AA Fever in general practice, I: frequency and diagnosis Fam Pract 1992;9263-269.
5. Baucher RGreen-Hernandez CedSingleton JKedAronzon DZed Fever: approach to the febrile child Primary Care Pediatrics. Philadelphia, Pa Lippincott Williams & Wilkins2001;343- 357
6. Guton H Human Physiology and Mechanisms of Disease 6th ed. Philadelphia, Pa WB Saunders1997;
7. Baraff LJBass JWFleisher GR et al. Agency for Health Care Policy and Research, Practice guideline for management of infants and children 0 to 36 months of age with fever without source Ann Emerg Med 1993;221198- 1210
8. Mukherjee PK. Quality Control of Herbal Drugs, 2nd Edition, Business Horizons, 2007; 2-14.
9. Kokate CK. Ed. Practical Pharmacognosy, 4th Edn., *Vallabh Prakashan*: 1994; 112:120.
10. Arpana Gaur Mishra, Richa Singh, Neha Patil, Geeta Parkhe. Determination of total phenolic, flavonoid content, antioxidant and antimicrobial activity of *gloriosa superba* seed extract. Asian Journal of Pharmaceutical Education and Research. 2017, 6(2):12-17.
11. Geeta Parkhe, Deepak Bharti. *In vitro* antioxidant activity, total phenolic and flavonoid contents of hydroalcoholic extract of leaves of *Lagerstroemia Parviflora* Roxb. Journal of Drug Delivery & Therapeutics. 2019; 9(4):708-711.
12. Teotino UM, Friz LP, Gandini A, Bella DD. 1963. Thio derivative of 2,3-dihydro-4H-1,3-benzoxazin-4-one syntheses and pharmacological properties. J Med Chem 6: 248–250.