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PHYTOCHEMICAL SCREENING AND ANTINOCICEPTIVE ACTIVITY OF *MIMOSA DIPLOTRICHA* LEAVES

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Keywords:

Antinociceptive activity, *Mimosa diplotricha*, Phytochemical analysis, Acetic acid induced writhing method, Tail immersion method

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ABSTRACT: Though possessing a lot of ethnopharmacological use, *Mimosa diplotricha* did not explore thoroughly for its bioactivity & the phytoconstituents responsible for its bioactivity. The purpose of the current work was to conduct phytochemical screening and antinociceptive activity of methanolic extract and its different fractions of *Mimosa diplotricha* leaves. Phytochemical screening of methanolic extract revealed the presence of alkaloids, carbohydrates, saponins, glycosides, phytosterols, phenols, flavonoids, proteins and lipids in *Mimosa diplotricha* leaves. The antinociceptive activity was assessed by using acetic acid induced writhing method and tail immersion method at two different doses using Swiss-albino mice as an animal model. In the mice model the methanolic extract and its n-hexane fraction showed Significant peripheral-antinociceptive activity at a dose of 400 mg/kg body weight with percentage of inhibition of acetic acid-induced writhing 56.25% ($P<0.001$) and 51.62% ($P<0.001$) respectively compared to the standard diclofenac sodium (62.50%, $P<0.001$) group. The antinociceptive effect of methanolic extract and its n-hexane fraction of *Mimosa diplotricha* at the 400 mg/kg and 200mg/kg dose level was also found to be significant ($P<0.001$ with 59.35% and 58.94% elongation of reaction time respectively) at 60 minutes in the tail immersion model compared to the standard morphine (64.17%, $P<0.001$) group. By these results, we believe that it would be worthwhile expanding these studies to include the potential source in the treatment of pain.

INTRODUCTION: Pain has been officially defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. It is always a warning signal and primarily protective but often causes a lot of discomforts and lead to many adverse effects [1, 2]. Analgesics relieve pain as a symptom, without affecting its cause. They are used when the noxious stimulus (evoking the pain) cannot be removed or as adjuvant to more etiological approach to pain [3].

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Other than their beneficial effects, these drugs were found to have different side effects such as gastrointestinal disturbances, renal damage, respiratory depression, and possible dependence in patients. Therefore, there is a need to develop new more effective analgesic agents with minimum side effects from natural sources [4].

Mimosa diplotricha is a species of a leguminous woody shrub which is an invasive species. It is commonly known as the giant sensitive plant, giant false sensitive plant, or nila grass. It is extensively found in South and South-East Asia, the Pacific Islands, northern Australia, South and Central America, the Hawaiian Islands, parts of Africa, Nigeria and France [5, 6]. It is well known for its rapid plant movement.

Like some other plant species, it undergoes changes in leaf orientation termed "sleep" or nyctinastic movement. The foliage closes during darkness and reopens in light [7]. It has much ethno-pharmacological uses like anti-convulsant, constipating agent, an antidiabetic agent, *etc*. and are used in the treatment of Urinary tract infections, fever, jaundice and inflammation [8, 9].

Earlier studies have shown that the extract of the plant possesses antioxidant activity [10], Cytotoxic activity [11] and antimicrobial activity [12]. As the plant is used in the treatment of fever in folkloric medicine and from our extensive literature survey, we found no antinociceptive studies on this valuable medicinal plant; therefore, the present study has been undertaken to investigate chemical constituents & the antinociceptive activity of methanolic extract and its different fractions of *Mimosa diplotricha* leaves.

MATERIALS AND METHODS:

Solvents and Chemicals: Analytical and laboratory grade (*e.g.,* SIGMA, E. Merck or BDH) solvents and chemicals were used in most of the experiments. Analytical and laboratory grade of Methanol, n-hexane, Carbon tetrachloride, Chloroform, Petroleum ether and others are used in extraction. Other reagents like Mayer's Reagent, Wagner's Reagent, Copper sulphate, Sulfuric acid, Potassium tartrate, Cupric sulphate, Sodium citrate, Anhydrous sodium carbonate, Pure –naphthol, Acetic anhydride, Hydrochloric acid, Lead acetate, Conc. Nitric acid, Glacial acetic acid, Iodine, *etc*. are used for Phytochemical screening. All were of the analytical grade.

Plant Collection and Authentication: The plant material, *Mimosa diplotricha* (leaf), was collected from Rangamati, hill tracks of Chittagong, Bangladesh on 26th January 2018. The plant was identified and authenticated by Dr. Shaikh Bokhtear Uddin, Taxonomist and Associate Professor of the Department of Botany, University of Chittagong, Bangladesh. A voucher specimen (accession number ACCU-2018/07) that contains the identification characteristics of the plant has been preserved for future reference.

Preparation of Plant Extract: After washing the Plant samples were first sun-dried for several weeks, crushed by hands and dried again. Then the crushed leaves were ground into coarse powder in the Phytochemical Research Laboratory, Biological Faculty, the University of Chittagong using high capacity grinding machine. About 800 gm. of the powdered material was taken in a clean, round-bottomed flask (5 Liters) and soaked in 3.5 liters of methanol for 15 days. The extract was filtered through Whatman filter paper number 1 and concentrated on a rotary evaporator (RE 200, Bibby Sterling Ltd., England) at 40 °C under reduced pressure.

The concentrated extract was finally evaporated to dryness on a water bath which afforded 28 g of crude extract. Then Solvent-solvent partitioning of crude methanolic extract was done by 4 different solvents (Petroleum ether, Carbon tetrachloride, Chloroform, n-hexane) using the protocol designed by Kupchan [13] and modified by Van Wagenen.

Phytochemical Analysis: Qualitative phytochemical tests for the identification of alkaloids, carbohydrates, flavonoids, phytosterols, glycosides, proteins & amino acids, gum and mucilages, lipids, volatile oil, terpenes, fixed oils and fats, saponins, phenols and tannins were carried out for the methanolic extract by the method described by Harborne and Sazada *et al*. [14, 15]

Experimental Animals: Swiss-albino mice (*Mus musculus*) of either sex, aged 4-5 weeks, weighing 20-25 gm each obtained from the BCSIR laboratories, Chittagong were used for the experiment. They were acclimatized to laboratory condition before the start of experiment. The animals were maintained in polycarbonate cages (23 ± 2 °C, relative humidity 60-70%, 12: 12 h light: dark cycle) and fed with a standard laboratory diet and water *ad libitum*. All animal experimentations were maintained and carried out according to the protocol approved by the Institutional Ethics Committee (IAEC, Reference no IIUC/AE 04).

Acute Toxicity Studies: Acute toxicity study was conducted, and the LD_{50} for each of the extract was determined, and one-tenth of the extract dose (LD_{50}) was selected as the maximum dose for the evaluation of Antinociceptive activity.

Assessment of *in-vivo* Antinociceptive Activity:

Acetic Acid-Induced Writhing Method: The acetic acid induced writhing method is a peripheral Antinociceptive behavioral observation assessment method that demonstrates a noxious stimulation in mice. The test consists of injecting 0.7% (v/v) acetic acid solution intraperitoneally and then observing the animal for writhing (specific contraction of the body) [16]. In this method, mice were divided into twelve groups of five each. The animals were pretreated with drugs 40 min before the induction of writhing. The animals received the standard drug Diclofenac (50 mg/kg, i.p) which served as reference standard. Antinociceptive activity of methanolic extract and its different fractions of *Mimosa diplotricha* (200 mg/kg & 400 mg/kg, p.o) was assessed by counting the number of writhes induced by 0.7% acetic acid (0.1 ml / 10 gm, i.p). The number of writhes per animal was counted for the next 15 min. The percentage protection against acetic acid was calculated using the following formula [17].

% inhibition = {(No. of writhes in control group - No. of writhes in test group) × 100} / No. of writhes in control group

Tail Immersion Method: Tail immersion test is performed to evaluate the central Antinociceptive property of methanolic extract and its different fractions of *M. diplotricha* leaves. It was designed based on the effect of central acting analgesic drugs in increasing the reaction time of mice in response to hot water [18]. Before analgesic experiment, the animals were screened for the sensitivity test by immersing the tail of the mice gently in hot water maintained at 55°C-55.5°C. The animal immersing the tail from hot water within 5 secs was selected for the study. Sixty experimental animals were randomly selected and divided into twelve groups denoted as Group-I, group-II, group-III(A-B), group- IV(A-B), group V(A-B) and group VI(A-B) consisting of 5 mice in each group. Each group received a particular treatment i.e. control, standard and different dose (200 mg/kg & 400 mg/kg, p.o) of the methanolic extract, pet ether, carbon tetrachloride, chloroform and n-hexane soluble fraction of methanolic extract respectively. After administration of the drugs, the reaction time was measured at 0, 30, 60, 90 & 120 min. The central antinociceptive activity of the test samples was compared with respect to the standard.

Elongation (%) = (Latency of Test-Latency of control) / Latency of test × 100

Statistical Analysis: Results of the study were represented by mean ± SEM (Standard Error Mean). Data were analyzed by one-way ANOVA followed by Dunnett's t-test and P values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION: Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack [19]. They are not essential nutrients and are not required by the human body for sustaining life, but have important properties to prevent or to fight some common diseases [20]. Because of this property; many studies have been undertaken to reveal the health benefits of phytochemicals. In the present study, the preliminary phytochemical evaluation of methanolic extract of *Mimosa diplotricha* leaves confirmed the presence of alkaloids, carbohydrates, saponins, glycosides, phytosterols, phenols, flavonoids, proteins and lipids **Table 1**.

Alkaloids, Carbohydrates, Saponins & Phytosterols were present in high concentration whereas Phenols & Flavonoids were in moderate concentration and Glycosides, Proteins and amino acids & Lipids were found in low concentration. On the other hand; Tannins, Gum and Mucilages, fixed oils and Fats, Volatile oil & Terpenes were found absent in this present study. Different chemical groups are responsible for exhibiting the different pharmacological effect. Identification for different chemical group reveals the possible pharmacological effect of the crude extract.

Alkaloids are phytochemicals that contain nitrogen and are derived from various amino acids. Alkaloids are known to be anti-arrhythmic effects, antihypertensive effects, anticancer and antimalarial activity [21-23]. Saponins are plant compounds that exhibit hypocholesterolemic, immunostimulant, hypoglycemic effect and anticarcinogenic properties [24]. Phytosterols inhibit absorption of cholesterol in the intestines [25]. Flavonoids have been reported to have anti-hyperglycemic effect [26].

TABLE 1: QUALITATIVE ANALYSIS OF THE PHYTOCHEMICALS OF METHANOLIC EXTRACT OF *MIMOSA DIPLOTRICHA*

Chemical Constituent	Results
Alkaloids	+++
Carbohydrates	+++
Glycosides	+
Saponins	+++
Tannins	-
Phenols	++
Gum and Mucilages	-
Flavonoids	++
Fixed oils and Fats	-
Proteins and amino acids	+
Phytosterols	+++
Lipids	+
Volatile oil	-
Terpenes	-

Symbol +++: present at high concentration; ++: present at moderate concentration; +: present at mild concentration; and -: absent of phytochemicals

Screening of *in-vivo* Antinociceptive Activity of *Mimosa diplotricha*:

Acetic Acid-Induced Writhing Test: The test was performed by taking the methanolic extract and other partitioning fractions at doses of 200 mg/kg and 400 mg/kg body weight. The result was found statistically significant.

The methanolic extract of *M. diplotricha* dose-dependently induced a significant (P<0.001) decrease in the number of writhes with 43.98% and 56.25% of inhibition at the dose of 200 mg/kg and 400 mg/kg body weight, respectively when compared to the control untreated group which was comparable to that of the standard drug diclofenac sodium (62.50% inhibition, P<0.001) **Table 2**.

TABLE 2: ANTINOCICEPTIVE ACTIVITY OF METHANOLIC EXTRACT AND ITS DIFFERENT FRACTIONS OF *MIMOSA DIPLOTRICHA*

Animal Group	Number of Writhing (Mean ± SEM)	% of Inhibition of Writhing
Control	43.2 ± 2.84	0
Standard	16.2 ± 0.92***	62.50
PEF 200	35.7 ± 1.98*	17.36
PEF 400	28.6 ± 1.11***	33.80
CTF 200	35.1 ± 1.82**	18.75
CTF 400	32.8 ± 1.47***	24.07
NHF 200	27.7 ± 1.08***	35.88
NHF 400	20.9 ± 0.93***	51.62
CHF 200	38.7 ± 1.31	10.42
CHF 400	36.5 ± 1.31*	15.50
ME 200	24.2 ± 1.19***	43.98
ME 400	18.9 ± 1.35***	56.25

Note: PEF = Pet ether Fraction, CTF = Carbon tetrachloride Fraction, NHF = n-hexane Fraction, CHF = Chloroform Fraction, ME = Methanolic Extract.

The partitioning fraction such as n-hexane soluble fraction at the dose of 400 mg/kg body weight exhibited a significant antinociceptive activity (51.62% inhibition of writhing). The pet ether, carbon tetrachloride and chloroform-soluble fractions at the dose of 200 and 400 mg/kg body weight exhibited lower antinociceptive activities (17.36, 33.80; 18.75, 24.07; and 10.42, 15.50% inhibition of writhing, respectively) and the actions were found to be significant (P<0.001) and dose-dependent **Table 2**.

Tail Immersion Method: In Tail immersion method the methanolic extract of *M. diplotricha* produced 46.77% (P<0.01), 59.35% (P<0.001), 50.21% (P<0.001) and 43.87% (P<0.01) & 43.56% (P<0.01), 54.78% (P<0.01), 50.21% (P<0.001) and 42.81% (P<0.01) elongation of reaction time at 30, 60, 90 and 120 min, respectively after oral administration at the 400 mg/kg & 200 mg/kg dose level **Table 3**. The % elongation of reaction time was increased with time and attained the peak level at 60 min (59.35% elongation) which is closer to that of the standard analgesic morphine (64.17%). So, the methanolic extract increased the pain threshold significantly (P<0.001) during the period of observation which is comparable to morphine solution. The n-hexane soluble fraction of *M. diplotricha* produced 41.92% (P<0.05), 58.47% (P<0.001), 54.94% (P<0.001) and 50.48% (P<0.001) & 40.99% (P<0.05), 58.94% (P<0.001), 51.02% (P<0.001) and 48.94% (P<0.001) elongation of reaction time at 30, 60, 90 and 120 min, respectively after oral administration at the 400 mg/kg & 200 mg/kg dose level **Table 3** which is contribute to significant central antinociceptive activity. The remaining pet ether, carbon tetrachloride, chloroform soluble fraction of methanolic extract of *M. diplotricha* also showed the highest % elongation at 60 min.

The results showed that the antinociceptive effect of methanolic extract of *M. diplotricha* at the 400 mg/kg dose level was found to be significant in the tail immersion model as well as in the acetic acid-induced model and thus it appears this extract exhibit analgesia through a peripheral and central mechanism. At the 400 mg/kg dose level, the antinociceptive action of the n-hexane soluble fraction was found to be more potent in tail immersion method **Table 3** than the acetic acid

induced writhing method **Fig. 1** and thus we can conclude that this fraction exhibited antinociceptive action predominantly in the central mechanism. The other fractions also exhibited moderate anti-nociceptive action in both central and peripheral mechanism.

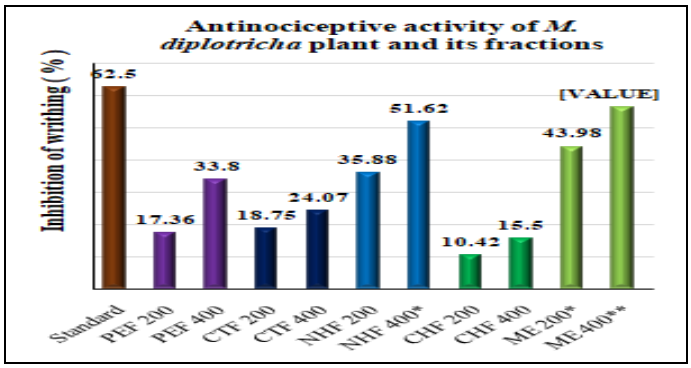

FIG. 1: ANTINOCICEPTIVE ACTIVITY OF METHANOLIC EXTRACT AND ITS DIFFERENT PARTITIONING FRACTIONS OF *MIMOSA DIPLOTRICHA*. *indicates considerable peripheral analgesic activity.

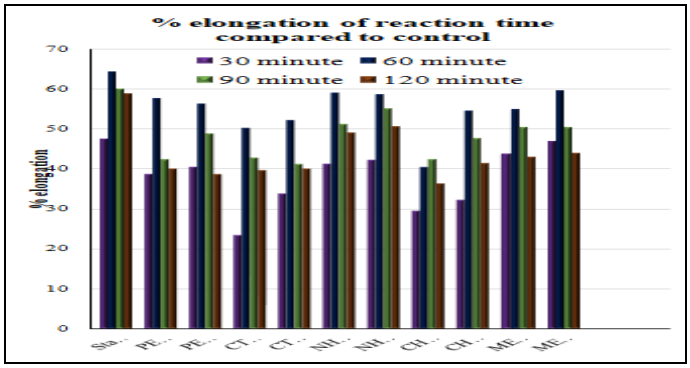

FIG. 2: ANTINOCICEPTIVE ACTIVITY OF *MIMOSA DIPLOTRICHA* BY TAIL IMMERSION METHOD. Each value represents the mean ± SEM. (n= 5). One- way ANOVA followed by Dunnett's t test. **P<0.001, *P<0.05 compared with control.

TABLE 3: EVALUATION OF ANTINOCICEPTIVE ACTIVITY OF METHANOLIC CRUDE EXTRACT AND DIFFERENT FRACTIONS OF *MIMOSA DIPLOTRICHA* BY TAIL IMMERSION METHOD

Treatment	Reaction times in seconds at time ± SEM and % Elongation				
	Pretreatment	30 min	60 min	90 min	120 min
Control	3.72 ± 0.31	4.78 ± 0.42	3.26 ± 0.24	3.60 ± 0.28	3.62 ± 0.26
Standard	3.01 ± 0.11	9.08 ± 1.19** (47.36)	9.10 ± 1.26*** (64.17)	8.96 ± 0.95 (59.82)	8.74 ± 1.07*** (58.58)
PEF200	3.34 ± 0.31	7.76 ± 0.69* (38.40)	7.65 ± 0.32*** (57.38)	6.23 ± 0.74** (42.21)	6.02 ± 0.27* (39.87)
PEF400	3.17 ± 0.56	7.99 ± 0.47* (40.17)	7.43 ± 0.32*** (56.12)	7.01 ± 0.25*** (48.64)	5.89 ± 0.62* (38.54)
CTF200	2.90 ± 0.48	6.23 ± 0.88 (23.27)	6.52 ± 0.81* (50.00)	6.27 ± 0.41** (42.58)	5.98 ± 0.48* (39.46)
CTF400	3.20 ± 0.40	7.19 ± 1.03 (33.52)	6.78 ± 0.45** (51.92)	6.11 ± 0.20** (41.08)	6.01 ± 0.31* (39.77)
NHF200	3.80 ± 0.28	8.10 ± 0.39* (40.99)	7.94 ± 0.65*** (58.94)	7.35 ± 0.44*** (51.02)	7.09 ± 0.59*** (48.94)
NHF400	3.40 ± 0.20	8.23 ± 0.99* (41.92)	7.85 ± 0.43*** (58.47)	7.99 ± 0.40*** (54.94)	7.31 ± 0.59*** (50.48)
CHF200	3.00 ± 0.34	6.76 ± 0.33 (29.29)	5.45 ± 0.39 (40.18)	6.23 ± 0.17** (42.21)	5.67 ± 0.43 (36.15)
CHF400	3.40 ± 0.49	7.04 ± 0.58 (32.10)	7.13 ± 0.17** (54.28)	6.85 ± 0.17*** (47.44)	6.15 ± 0.30* (41.14)
ME200	3.10 ± 0.67	8.47 ± 0.72** (43.56)	7.21 ± 1.20** (54.78)	7.23 ± 0.37*** (50.21)	6.33 ± 0.51** (42.81)
ME400	3.30 ± 0.16	8.98 ± 0.38** (46.77)	8.02 ± 0.55*** (59.35)	7.23 ± 0.43*** (50.21)	6.45 ± 0.49** (43.87)

Note: PEF = Pet ether Fraction, CTF = Carbon tetrachloride Fraction, NHF = n-hexane Fraction, CHF = chloroform Fraction, ME = Methanolic Extract.

CONCLUSION: This study has explored the various phytochemicals, including alkaloids, carbohydrates, saponins, glycosides, phytosterols, phenols, flavonoids, proteins and lipids present in the leaves of *M. diplotricha* for the first time. The results of this study also demonstrate that the extracts of *M. diplotricha* has antinociceptive property both in central and peripheral mechanism and they show a significant increase in reaction time created by thermal stimuli & inhibition of writhing created by acetic acid. Afterward, the further aim of this study is too detailed investigations to isolate and identify the active compounds present in the plant extract, and their efficacy needs to be done. It will help in the development of novel and safe drugs for the treatment of different types of disorders.

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CONFLICT OF INTEREST: We declare no conflict of interest for this research.

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