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Non-clinical and pre-clinical pharmacological investigations of *Mimosa diplotricha*

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ABSTRACT

The present study aims to screen pharmacological and phytochemical screenings of the *Mimosa diplotricha*. For this the ethanolic crude extract of *M. diplotricha* (EMd) was undergone for checking antioxidant, anti-inflammatory and membrane stabilizing, anti-diarrheal and clotlysis activities followed by a preliminary phytochemical screening. Results suggest that, the EMd concentration-dependently exhibited antiradical (DPPH: 1,1-diphenyl-2-picrylhydrazyl) and anti-inflammatory activities. The EMd also exhibited promising anti-diarrheal effect in Swiss mice, while a negligible clotlysis activity was observed in clotted human blood. Additionally, the phytochemical screening of the crude plant extract revealed the presence of alkaloids, glycosides, flavonoids and reducing sugars. In conclusion, *M. diplotricha* may be a good source of antioxidant and anti-diarrheal agents. Further researches are recommended for the isolation and characterization of its phytochemicals.

Keywords: antioxidant; anti-inflammatory; anti-diarrheal; *Mimosa diplotricha*.

INTRODUCTION

Medicinal plants are considered as a rich resource of ingredients and are being used as a potential source of drugs or drug-like substances. Notably, plants have a great leader in the discovery and development of modern drugs.

Mimosa is a genus of about 400 species of herbs and shrubs, in the subfamily Mimosoideae of the legume family Fabaceae*. Mimosa diplotricha* is a shrub or sprawling annual vine which may also behave as a perennial and is native to the neotropics though it has now become widespread throughout the wet tropics and subtropics, and is usually a very invasive species wherever introduced. In a recent study, three water-soluble polysaccharides isolated from *M. diplotricha* leaf extract demonstrated to have promising antioxidant capacities, where superoxide ($O_2^{\bullet-}$), 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS$^{\bullet+}$), hydroxyl ($^{\bullet}$OH), nitric oxide (NO$^{\bullet}$), N,N-dimethyl-p-phenylenediamine (DMPD$^{\bullet+}$) radical scavenging activities were observed. Additionally, the ferric ion (Fe^{3+}) reducing, ferrous ion (Fe^{2+}) chelating and inhibition of lipid peroxidation activities were also demonstrated (RANA et al., 2014). Previously, four new 5-deoxyflavones, namely 2',5'-dihydroxy-3,7,8,4'-tetramethoxyflavone (1), 3'-hydroxy-3,7,8,4'-tetramethoxyflavone (2), 2'-hydroxy-7,4',5'-trimethoxyflavone (3) and 4-hydroxy-3,10,11-trimethoxyisochromeno-[4,3-b]-chromen-7(5H)-one (4) was also reported for their promising cytotoxic effects on the human cancer cell lines: A549, AGS, HT-29, and PC3. Compound 2 and 5″-methoxyhydnocarpin-D isolated from this plant also exhibited potent antiproliferative activity (LIN et al., 2011). Otherwise, YUSUF et al. (2009) demonstrated that, *M. diplotricha* is used by the tribal traditional medicine practitioners for treating diarrhea and various infectious diseases.

Our present study is undergone to investigate the antioxidant, anti-inflammatory, anti-diarrheal and anti-atherothromobosis activities by adopting some non-clinical methods along with a preliminary screening for phytochonstituents.

MATERIALS AND METHODS

2.1. Plant collection and identification

For the investigation, fresh leaves of *M. diplotricha* were collected from the Chittagong district in Bangladesh in the month of October. The plant material was then identified by the taxonomist (without voucher specimen), from Bangladesh Forest Research Institute Herbarium (BFRIH), Chittagong, Bangladesh.

2.2. Extraction

After collection, the plant materials were subjected for shade drying (temperature not exceeding 50 °C) and pulverized to obtain a course powder. Approximately 300 gm of powdered material was extracted with 95% ethanol at 10 h by using a Soxhlet extractor (Quickfit, England) and filtered through a cotton plug followed by the Whatman filter paper (no. 1), and concentration by evaporating the solvent with a rotary evaporator (bellow 50 °C). The yield of the EMd was 15.61%.

2.3. Screening for antioxidant activity

(DPPH scavenging assay)

The antioxidant activity of the EMd was determined by the method described earlier ISLAM et al, (2016a). Briefly, a stock solution of the plant extract was prepared in vehicle (0.05% Tween 80 dissolved in 0.9% NaCl solution), following to reconstituting a concentration range of 10 to 100 µg/ml. In the diluted sample (0.3 ml), 2.7 ml of 0.004 % DPPH ethanolic solution was added. Then the contents were mixed properly, allowed to stand at dark for 30 min to complete the reaction and absorbance was taken using a spectrophotometer at 517 nm. A similar concentration of ascorbic acid (AA) was served as the positive control, while only 0.3 ml vehicle was added to the DPPH solution for the negative control (NC). The blank contained no sample. The DPPH radical scavenging potential was calculated using the following equation 1:

$$\textbf{\%}\ \text{inhibition of DPPH radical} = [(A_{br} - A_{ar})/A_{br}] \times 100 \quad \text{(Equação 1)}$$

where, A_{br} is the absorbance of before and A_{ar} is the absorbance of DPPH free radicals after reaction.

2.4. Screening for *in-vitro* anti-inflammatory activity

(Egg albumin (EAL) test)

The anti-inflammatory (EAL; *in vitro*) of EMd was carried out according to HOSSAIN et al. (2013). Briefly, 1% egg albumin (EAL) was constituted in phosphate buffer saline solution (PBS, pH 7.4). The assay mixture contains 1 ml phosphate buffer (pH 7.4, 0.15 M), 2 ml hypo-saline (0.36%). 0.5 ml of EAL was mixed with 0.5 ml of the test sample within a concentration range 125 to 500 µg/ml. Acetyl salicylic acid (ASA) and 0.05% Tween 80 dissolved in 0.9% NaCl solution were taken as positive and negative (NC) controls. After incubation at 37°C for 30 min, the reaction mixtures were centrifuged and the supernatant was collected for spectrophotometric analysis at 560 nm. Activity was measured by the following

equation 2.

Inhibition (%) = 100 - [(absorbance of test solution) ÷ (absorbance of control) × 100] (Equação 2)

2.5. Screening for anti-diarrheal activity

(Castor oil-induced diarrheal model)

The anti-diarrheal activity of the EMd was determined by the method described in NUR et al. (2015). Briefly, the animals were all screened initially by giving 0.4 ml of castor oil and only those showing diarrhea were selected for the final experiment. Swiss mice (both sex; 25-30 gm body weight or two months old) were divided into control, positive control and a test group containing five animals in each. The control group received 0.05% Tween-80 dissolved in 0.9% NaCl solution (10 ml/kg), while the positive control and test groups received loperamide EMd at 3 and 500 mg/kg, respectively. All the treatments were administered *via* oral gavage (p.o.). Each animal was placed in an individual cage, the floor of which was lined with blotting paper, which was changed every hour. Diarrhea was induced by oral administration of castor oil (0.4 ml) to all mice, 30 min after the above treatments. During the observation period (4 h), the latency period (first diarrheal defecation time) and frequency (number of defecation) were counted manually. Percent inhibition of defecation in mice was calculated by using the following equation 3:

% inhibition = {(Mo–M)/Mo} × 100 (Equação 3)

where, Mo =Mean defecation of control and M = Mean defecation of test sample.

2.6. Screening for anti-atherothrombotic activity

(Clot lysis test in human blood)

The thrombolytic activity of the EMd was evaluated by the method of SULTANA et al. (2012) using streptokinase (SK) 100 I.U and previously said NC, respectively. Briefly, blood was collected from 10 healthy volunteers and distributed into pre-weighed (W_1) micro-centrifuge tubes (0.5 ml/tube) and incubated at 37°C for 45 min and then weight (W_2) was taken. The weight of clotted blood (ΔW) was taken by subtracting the pre-weight and the weight of clotted blood containing tube. Then 100 µl of the treatments were added to the clot containing tubes marked. The EMd was tested at a concentration of 500 µg/ml. Similarly, 100 µl of streptokinase (100 IU/tube) and 100 µl of NC were added to the controls marked tubes. All the tubes were then incubated at 37°C for 90 min. After incubation, fluid released was removed carefully without disrupting the clot, and tubes were again weighed for getting the weight variation among the pre-weight and final weight (W_3) that was achieved for clot lyses (thrombolysis).

2.7. Preliminary screening for phytoconstituents

The phytochemical group test was screened according to the earlier described method by HARBORNE (1973) and SOFOWORA (1993) (not discussed).

2.8. Statistical analysis

Values are mean ± SD (standard deviation) and percentages. The data were analyzed by means of analysis of variance (ANOVA) followed by *t*-Student–Newman–Keuls's as post-hoc test using the GraphPad Prism software (version 6.0) with 95% confidence interval at $p < 0.05$.

RESULTS AND DISCUSSION

Medicinal plants have been used to prevent and treat various health problems. Hence there is considerable incentive to discover new chemical agents from plants. Considering the vast area of potentiality of plants as sources of drugs and taking into account the local traditional uses, a systematic investigation was undertaken to screen the phytochemical and biological activity of *M. diplotricha* belonging to the family of Fabaceae.

In the radical scavenging assay, highest inhibition (61.66 ± 1.08%) of the DPPH• was observed with the concentration tested 100 µg/ml. Although a concentration-dependent antiradical activity was observed with EMd, the activity was lower than that of the standard (AA) treated group (Table 1).

Table 1: Antiradical activity of EMd and the controls by DPPH scavenging capacity.

Concentration (µg/ml)	Percent inhibition of DPPH radical	
	EMd	AA
10	20.56 ± 1.08*	30.21 ± 1.21*
20	30.03 ± 1.08*	42.32 ± 1.08*
40	40.22 ± 0.58*	60.09 ± 1.08*
60	47.73 ± 0.47*	69.93 ± 0.58*
80	58.75 ± 0.43*	80.41 ± 0.47*
100	61.66 ± 1.08*	94.48 ± 1.03*
IC_{50} (µg/ml)	**19.61 ± 0.27**	**21.80 ± 0.23**
CI	12.97 – 29.64	14.72 – 32.30
r^2	0.92	0.93
NC	2.01 ± 1.08	

**p <0.05 compared to NC (negative control: 0.5% Tween 80 dissolved in 0.9% NaCl solution); values are percentage of inhibition of DPPH• ± SD (n = 5); EMd: ethanol leaf extract of M. diplotricha; AA: Ascorbic acid; IC_{50}: half minimal inhibitory concentration.*

The DPPH test is widely used for the

preliminary assessment of antioxidant capacity of a vast number of substances due its rapidity, economy, and ease of test proceeding. In most cases, a precedent to other antioxidant capacity assays is followed by knowing the potentiality of the DPPH• scavenging capacity of any substances (ISLAM et al., 2016a). In our study, the EMd exhibited significant ($p < 0.05$) DPPH• scavenging capacity in comparison to the NC group.

In the *in vitro* anti-inflammatory test, EMd at concentration ranges 120-500 µg/ml, exerted an inhibitory effect in protein denaturation. Although a concentration-dependent augmented response was observed, but no sharp changes were seen between the test concentrations. The highest inhibitions of protein denaturation were 25.25 ± 1.21% and 46.08 ± 1.33% with the 500 µg/ml of EMd and the standard (ASA), respectively (Table 2).

Table 2: Anti-inflammatory activity of EMd and controls in egg albumin.

Conc. (µg/ml)	% inhibition of protein denaturation	
	EMd	ASA
125	22.30 ± 2.41*	31.37 ± 2.01*
250	23.53 ± 3.40*	38.73 ± 2.18*
500	25.25 ± 1.21*	46.08 ± 1.33*
IC_{50} (µg/ml)	**28.85 ± 0.68**	**82.28 ± 0.63**
CI	~	3.49 - 1938
r^2	0.89	0.94
NC	2.87 ± 1.09	

**p <0.05 compared to the NC (negative control: 0.5% Tween 80 dissolved in 0.9% NaCl solution); values are percentage inhibition ± SD (n = 5); EMd: ethanol leaf extract of M. diplotricha; ASA: acetyl salicylic acid.*

The egg albumin (EAL) test is a rapid, economic and sensitive test to evaluate anti-inflammatory potential of a substance, as the protein molecules are highly sensitive to the temperature chemical-induced damaging effects (HOSSAIN et al., 2013). In our study, in comparison to the NC and ASA groups, EMd-mediated albumin protestant effect may be a good indication of its anti-inflammatory capacity.

In the castor oil induced diarrheal mice, the EMd at a dose of 500 mg/kg significantly ($p < 0.05$) increased and latency period and decreased in faeces by 104.2 ± 5.59% and 1.80 ± 0.84%, respectively. Although, the activity was slightly lower than the standard drug loperamide, but it should be claimed as strong in comparison to the NC group (Table 3).

Table 3: Anti-diarrheal activity of EMd and controls in castor oil-induced diarrheal mice.

Treatment groups	Dose (p.o.)	Latency (min)	% inhibition of defecation	Faeces
NC	10 ml	1.40 ± 0.55	10.63	15.20 ± 2.28
Loperamide	3 mg/kg	138.8 ± 7.40*	98.00	1.20 ± 0.45*
EMd	500 mg/kg	104.2 ± 5.59*	89.53	1.80 ± 0.84*

**p <0.05 compared to the NC (negative control: 0.5% Tween 80 dissolved in 0.9% NaCl solution); values are mean ± SD (n = 5) and percentage; EMd: ethanol leaf extract of M. diplotricha.*

Castor oil is evident to split to liberate free fatty acid in the presence of lipase, bile, and water, with a limited hydrolysis. It acts *via* motor stimulation of the small intestine. The fluid character of the stool is due to the quick passage of the faeces (Iwao and Terada, 1962). On the other hand, substances having active hydroxyl group (-OH) may exert an antimicrobial effect, especially those are terpenoids and flavonoids (ISLAM et al., 2016b). *Salmonella*, *Shigella* and *Escherichia coli* are the known microbes that cause diarrhea in human and animals (NUR et al., 2015). However, having these types of compounds is also reported for potential antioxidant capacity (ISLAM et al., 2016a,b).

A clot inside the blood vessel may obstacle the passages of blood and nutrients, thus barrier in fulfilling oxygen and nutrients to the cells, necessary for normal metabolic processes (SULTANA et al., 2012). The formation of thrombus inside the blood vessel is also evident for various heart diseases including stroke, an event of sudden death. In the *ex-vivo* thrombolytic activity test, the EMd at 500 µg/ml caused a negligible lysis of the clotted blood inside the tubes. Data when compared with the NC and SK groups, demonstrated an insignificant clotlysis capacity (**Table 4**).

Table 4: Anti-atherothrombosis activity of EMd and controls in clotted human blood.

Treatments	Concentration/tube containing 500 µl of blood	Percentage of clotlysis
NC	100 µl	1.50 ± 1.58
SK	100 IU	81.08 ± 0.027*
EMd	500 µg/ml	4.59 ± 3.73

**p <0.05 compared to the NC (negative control:*

0.5% Tween 80 dissolved in 0.9% NaCl solution); values are mean ± SD; EMd: ethanol leaf extract of M. diplotricha; SK: streptokinase.

In our study, in the preliminary phytochemical investigation is suggesting that, the EMd possesses alkaloids, glycosides, flavonoids, and reducing sugar (Table 5).

Table 5: Phytochemical groups found in EMd.

Phytoconstituent		*Alkaloids*	*Glycosides*	*Steroids*	*Tannins*	*Falvonoids*	*Saponins*	*Reducing sugars*	*Gums*
Consequence	EMd	+ + +	+ + -	- -	- -	+ +	-	+	-

** (+) = presence; (-) = absence; number indicates number of test performed; EMd: ethanol leaf extract of M. diplotricha.*

The presence of polysaccharides and flavonoids in *M. diplotricha* leaf is also reported earlier along with strong antiradical, and cytotoxicity activities (LIN et al., 2011; RANA et al., 2014). This study demonstrating the presence of both chemical groups, thus a linking to its literature reports. Generally, the antioxidants are cytoprotective in nature. They scavenged the free radicals (or ROS/RNS: reactive oxygen/nitrogen species), coming from both external and internal sources inside a biological system. Additionally, they may power the body antioxidant systems (e.g. – superoxide dismutases, catalase, glutathione) to minimize the ROS. A number of such types of agents are also evident to exert anti-inflammatory effects, especially those are from ROS originated. Otherwise, antioxidants at high concentration may impart an antioxidant-induced 'pro-oxidative effect', known as protective effect. Although, it is commonly characterized as cyto-genotoxic event, but helpful to kill a number of pathogens. In this way, antioxidant, anti-inflammatory and cytotoxic potentials have linked one another. Thus the antioxidant, anti-inflammatory and antidiarrheal capacity of EMd may be connected to each other.

CONCLUSION

The results obtained in the present non-clinical studies are in agreement to the traditional uses as well as literature reports of uses of *M. diplotricha*. We suppose the observed results may link to the polysaccharides and flavonoids present in the leaf extract. The antioxidant, anti-inflammatory along with anti-diarrheal activity may be good indications of its cytoprotective, thus the health promotion usages. However, further researches are cordially welcomed.

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