

ANTI-INFLAMMATORY, ANTHELMINTIC & ANTIDIABETIC ACTIVITY OF AQUEOUS EXTRACT OF *MICROCOS PANICULATA* FRUITS

Aziz, M.A.¹; Rahman, S.²; Islam, T.²; Islam, T.²; Alam, A.S.²; Uddin, N.²; Hasan, N.²,
Hossain, M.M.²; Rana, M.S.²

¹Department of Pharmacy, Stamford University Bangladesh, 51, Siddeswari Road, Dhaka-1217, Bangladesh.

²Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.

[*abdullahaziz2010bdn@gmail.com](mailto:abdullahaziz2010bdn@gmail.com)

Abstract

Aqueous extract of *Microcos paniculata* fruits (FAE) was evaluated for its anti-inflammatory, anthelmintic & antidiabetic activity by using proteinase inhibitory assay, *Pheretima posthuma* model & α -amylase inhibitory assay. In case of proteinase inhibitory assay, FAE showed significant ($P < 0.05$) antiproteinase activity (41.05% proteinase inhibition at 250 $\mu\text{g/mL}$ with an IC_{50} of 285.47 $\mu\text{g/mL}$). Again, FAE at a dose of 50 mg/mL caused paralysis & death of *Pheretima posthuma* at 34.24 min and 55.25min respectively. Moreover, the extract demonstrated significant ($P < 0.05$) α -amylase inhibition with an IC_{50} value of 1367.56 $\mu\text{g/mL}$. The results obtained in the present study point out that FAE can be a possible source of anti-inflammatory, anthelmintic & antidiabetic agents.

Keywords: Anti-inflammatory, anthelmintic, antidiabetic, *Microcos paniculata*.

Introduction

Inflammatory disorders are characterized by excessive activation of phagocytes. Besides, generation of OH radicals and non free radicals species like H_2O_2 can lead to tissue damage [1].

Helminths may cause morbidity along with extensive social and economic scarcity among animals and humans. Moreover, they are responsible for the prevalence of anaemia, pneumonia, malnutrition and eosinophilia [2]. α -amylase enzyme hydrolyzes starches into oligosaccharides. Though conventional antidiabetic drugs inhibit this enzyme but they produce various side-effects [3]. *Microcos paniculata* L. (Family: Tiliaceae) is popularly known in Bangladesh as Fattashi or Kathgua. It is seemed as small tree or shrub and grown in Bangladesh, Sri Lanka, India, Indonesia, Myanmar, Malaysia, China, Andaman Islands, Vietnam, Cambodia and Thailand. Traditionally it is used to treat diarrhea, dyspepsia, fever, heat stroke, colds, wound healing, insect infestation and hepatitis. Due to alkaloid, the stem bark of this plant showed activity against the 2nd instar *Aedes aegypti* larvae. Piperidine alkaloids were separated from the leaves of this plant that revealed activity against the *Culex quinquefasciatus* larvae. Free-radical-scavenging activity was found from the stem of this plant. And the ethanolic extract of the leaves of this plant exhibited cytotoxic activity. In another study, it was found that *M. paniculata* roots demonstrated larvicidal and antimicrobial activity along with brine shrimp lethality [4]. However, methanolic extract of *M. paniculata* leaves also contained antidiarrheal activity [5]. Analgesic activity was found from the leaves [6]. In addition to, *M. paniculata* also showed nicotinic receptor antagonistic activities [7]. Therefore, in this study, the aqueous extract of *Microcos paniculata* fruits was subjected to evaluate the anti-inflammatory, anthelmintic & antidiabetic activity.

Materials and Methods

Plant Materials: Collection and Identification

M. paniculata fruits were collected from the Jahangirnagar University campus, Savar, Dhaka, Bangladesh in November, 2013, which were identified by Sarder Nasir Uddin, Principal Scientific Officer at the Bangladesh National Herbarium having the accession number 35348. A dried specimen was deposited in the herbarium.

Preparation of the Plant Extract

The extraction procedure was carried out by using

170 g of dried powdered fruits of *M. paniculata*. Fruits were washed by using running water and sterile distilled water & dried under shade for 7 days. Grinding mill (Model 2000 LAB Eriez®) was employed for powdering the dried fruits and passed through a 40-mesh sieve to get fine powder. 1.7 L water was used for extraction using soxhlet apparatus following hot extraction procedure. After that, filtration was done by Whatman No.1 filter papers. Hot air oven was utilized for drying the filtrate whose temperature was maintained at 40°C. The extraction yield of FAE was 6.90% (w/w). The extract was stored at 4°C using a refrigerator.

In vitro Anti-Inflammatory Study Proteinase Inhibitory Assay

The *in vitro* anti-inflammatory study was conducted by detecting proteinase inhibitory activity according to Oyedapo et al [8] and Kumarappan et al [9]. Briefly, trypsin (0.06 mg), 25 mmol/L tris-HCl buffer (1 mL, pH 7.4) and 1.0 mL aqueous solution of the aspirin (standard drug) or the fruit extract of *M. paniculata* (50–250 μ g/mL) were added to make the reaction mixtures, having the volume of 2 mL. After 5 minutes of incubation at 37 °C, 1 mL of 0.8% casein (w/v) was added and continually incubated for 20 min. The reaction was terminated by the addition of 2.0 mL 70% perchloric acid. Cloudy suspension was generated and was centrifuged later at 1109 x g for 10 min. Supernatant absorbance was found at 280 nm against the buffer using as blank. The percentage of proteinase inhibition and IC₅₀ values were calculated taking the average of triplicate values.

Proteinase inhibition (%) = $[1-A/B] \times 100$

Here, A = Absorbance of extract or standard drug;

B = Absorbance of control

Anthelmintic Assay

Pheretima posthuma Model

The method of Ajaiyeoba et al., 2001 was used for carrying out the anthelmintic assay with slight modifications [10]. *Pheretima posthuma* adult earthworm was used for conducting the assay due to its physiological and anatomical similarity with the roundworm parasite of human intestine [11, 12]. The groups of equal sized earthworms consisting of 6 earthworms in each group were released in 50 mL of sample with desired concentrations of 10, 25 and 50 mg/mL. Group of earthworms in 0.9% NaCl solution was used as control group and group of earthworms in piperazine citrate (10 mg/mL) used as standard. Observations were made for the time taken for paralysis and death of individual worm. Paralysis as

said to occur when no movement of any sort could be observed except the worms was shaken vigorously. Death was concluded when the worms did not move being shaken vigorously or when dipped in warm water at 50°C.

In vitro Antidiabetic Study α-Amylase Inhibitory Assay

This study was performed by a modified starch iodine protocol [13]. In short, 1 mL of plant extract or standard (Acarbose) of different concentrations (2, 1, 0.5 mg/mL) was taken in pre-labeled test tubes. A volume of 20 µL of α-amylase was added to each test tube and incubated for 10 min at 37°C. After the incubation, 200 µL of 1% starch solution was added to each test tube and the mixture was re-incubated for 1 hr at 37°C. Then 200 µL of 1% iodine solution was added to each test tube. Later, 10 mL distilled water was added separately. Absorbance of the mixture was taken at 565 nm. Sample, substrate and α-amylase blank were undertaken under the same conditions. Each experiment was done in triplicate. IC₅₀ values were calculated taking the average of triplicate values.

$$\% \alpha\text{-amylase inhibition} = [1 - (\text{SA} - \text{SBB}) - \text{SMB} / \text{AAB}] \times 100$$

SA=Sample absorbance, SMB=Sample blank, SBB=Substrate blank and AAB=α-Amylase blank.

Statistical Analysis

All results were expressed as mean ± S.E. (Standard Error). Statistical analyses for % proteinase & α-amylase inhibition activities were evaluated by one way analysis of variance (ANOVA) followed by Post hoc Tukey's HSD test through SPSS software (version 17; IBM Corporation, New York, USA). IC₅₀ values were calculated by linear regression equations through the usage of Microsoft Excel 2007 (Microsoft, Redmond, Washington, USA).

Results

In vitro Anti-Inflammatory Study Proteinase Inhibitory Assay

In the present study, FAE was found to possess significant ($P < 0.05$, vs aspirin group) antiproteinase activity (Table 1) when pair wise mean comparison was performed. Most importantly, the highest effect of FAE was observed at 250 µg/mL having 41.05% proteinase inhibition with an IC₅₀ of 285.47 µg/mL (Table 1 and 2).

Anthelmintic Assay

***Pheretima posthuma* Model**

The crude FAE revealed varying degree of anthelmintic activity; i.e. the extract exhibited not only paralysis but also death of worms at all the tested concentrations. It was also seen that at the concentration of 50 mg/mL the extract demonstrated shortest time for paralysis and death of earthworm. At this concentration, the FAE caused paralysis of *Pheretima posthuma* at 34.24 min and death at 55.25 min, while piperazine citrate (positive control) caused paralysis and death at 23.24 min and 36.93 min, respectively at 10 mg/mL. Thus it was also clear from the study (Table 3) that, the concentration of the extract has an inversely proportional relationship with the time of paralysis and death of worms.

In vitro Antidiabetic Study α-Amylase Inhibitory Assay

In the present study, FAE was found to possess inhibitory effects on starch break down shown in table 4. 1367.56 µg/mL was found as the IC₅₀ value of FAE, along with 58.75% α-amylase inhibition at 2000µg/mL (Table 4 & 5). But the standard drug acarbose showed the IC₅₀ value as 785.84 µg/mL (Table 5).

Discussion

Proteinases play role for creating arthritic reactions. Neutrophils are identified as a precursor of proteinase. During inflammatory reactions, tissue damage occurs by leukocytes proteinase. And proteinase inhibitors prevent this tissue damage. It was reported that many polyphenolic compounds like flavonoids, tannins, terpenoids, saponins are responsible for anti-inflammatory activities of many plants [14]. Phytochemical study showed that aqueous extract of *M. paniculata* fruits contained steroids that are phenolic in nature [15]. So there may be different mechanism/mechanisms for showing the anti-inflammatory activity of the aqueous extract of *M. paniculata* fruits. Tannins which are polyphenolic in nature are responsible for anthelmintic activities of medicinal plants. The anthelmintic effects of tannins are related with several mechanisms. Tannins cause death of parasites by hampering energy production in them through uncoupling oxidative phosphorylation or attachment with the glycoprotein on the cuticle of parasite [16]. Previous phytochemical study revealed that the aqueous extract of *M. paniculata* fruits did not contain tannins [15] though the extract showed anthelmintic activity. The exact mechanism for its

activity is unknown to us till now. To find out the reason/reasons of its anthelmintic activity we can fractionate the crude extract. Moreover, it is desirable to isolate the active compound of this extract which showed the anthelmintic activity.

Alpha amylase breaks polysaccharide into monosaccharide [17]. Hence, diabetes can be controlled by inhibiting alpha amylase enzyme which reduces the absorption of glucose from starch [18]. Aqueous extract of *M. paniculata* fruits exerted its antidiabetic activity that may due to its inhibitory effect on alpha amylase enzyme.

Conclusion

Anti-inflammatory, anthelmintic & antidiabetic tests were used as preliminary study in search for natural anti-inflammatory, anthelmintic & antidiabetic agents. Based on the results presented in this work, aqueous extract of *M. paniculata* fruits can offer an opportunity for new compounds. Further chemical studies are now under way to isolate the compounds responsible for these activities.

Acknowledgement

The authors would like to thank Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh for providing facilities throughout the work.

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Table 1. Proteinase inhibition activity of the standard drug and FAE.

Test Samples	% Proteinase inhibition activity				
	50 µg/mL	100 µg/mL	150 µg/mL	200 µg/mL	250 µg/mL
Aspirin	52.86±0.42 ^a	67.86±0.45 ^a	81.17±0.52 ^a	88.95±0.08 ^a	97.33±0.17 ^a
FAE	9.62±0.35 ^b	15.41±0.57 ^b	29.81±0.21 ^b	38.50±0.45 ^b	41.05±0.17 ^b

Values are presented as mean±S.E., n=3. Tukey HSD test was performed for the pair wise mean comparison. Values along the same column with different superscripts a and b are significantly different from one another (P<0.05).

Table 2. IC₅₀ of the standard drug and aqueous extract of *M. paniculata*.

Test Samples	IC ₅₀ (µg/mL)
Aspirin	24.46±1.80
FAE	285.47±0.57

Values are presented as mean±S.E., n=3.

Table 3. Evaluation of anthelmintic activity of FAE.

Group	Doses (mg/mL)	Time for paralysis (min)	Time for death (min)
Control	10	No paralysis	No death observed
Standard	10	23.24± 0.18	36.93 ± 0.64
	10	127. 24±0.92	135.19±0.62
FAE	25	73.87±0.96	122.12±0.37
	50	34.24±0.60	55.25±0.25

Values are presented as mean±S.E., n=6 worms.

Table 4. % of α-amylase inhibition of standard drug and FAE.

Group	%of α-amylase inhibition		
	500 µg/mL	1000 µg/mL	2000 µg/mL
Standard	42.48±0.25 ^a	58.13±0.58 ^{ab}	74.03±1.02 ^{ac}
FAE	33.64±0.07 ^{bc}	50.43±0.24 ^b	58.75±0.09 ^{ab}

Values are presented as mean±S.E., n=3. Tukey HSD test was performed for the pair wise mean comparison. Values along the same column and row with different superscripts a, b, ab, ac & bc are significantly different from one another (P<0.05).

Table 5. IC₅₀ of standard drug and FAE of *M. paniculata*.

Test Samples	IC ₅₀ (µg/mL)
Standard	785.84±11.86
FAE	1367.56±2.73

Values are presented as mean±S.E., n=3.