Research Article

Qualitative phytochemical screening and evaluation of anti-inflammatory, analgesic and antipyretic activities of *Microcos paniculata* barks and fruits

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ABSTRACT

**OBJECTIVE:** The main objectives of this study were to qualitatively evaluate the profile of phytochemical constituents present in methanolic extract of *Microcos paniculata* bark (BME) and fruit (FME), as well as to evaluate their anti-inflammatory, analgesic and antipyretic activities.

**METHODS:** Phytochemical constituents of BME and FME were determined by different qualitative tests such as Molisch’s test, Fehling’s test, alkaloid test, frothing test, FeCl₃ test, alkali test, Salkowski’s test and Baljet test. The anti-inflammatory, analgesic and antipyretic activities of the extracts were evaluated through proteinase-inhibitory assay, xylene-induced ear edema test, cotton pellet-induced granuloma formation in mice, formalin test, acetic acid-induced writhing test, tail immersion test and Brewer’s yeast-induced pyrexia in mice.

**RESULTS:** *M. paniculata* extracts revealed the presence of carbohydrates, alkaloids, saponins, tannins, flavonoids and triterpenoids. All of the extracts showed significant (P<0.05, vs aspirin group) proteinase-inhibitory activity, whereas the highest effect elicited by plant extracts was exhibited by the BME (75.94% proteinase inhibition activity) with a half-maximal inhibitory concentration (IC₅₀) of 61.31 μg/mL. Each extract at the doses of 200 and 400 mg/kg body weight showed significant (P<0.05, vs control) percentage inhibition of ear edema and granuloma formation. These extracts significantly (P<0.05, vs control) reduced the paw licking and abdominal writhing of mice. In addition, BME 400 mg/kg, and FME at 200 and 400 mg/kg showed significant (P<0.05, vs control) analgesic activities at 60 min in the tail immersion test. Again, the significant (P<0.05, vs control) post-treatment antipyretic activities were found by BME 200 and 400 mg/kg and FME 400 mg/kg respectively.

**CONCLUSION:** Study results indicate that *M. paniculata* may provide a source of plant compounds with anti-inflammatory, analgesic and antipyretic activities.

**Keywords:** phytochemical screening; agents, anti-inflammatory; analgesics; antipyretic agents; *Microcos paniculata*; medicine, herbal


1 Introduction

Many traditional medicine systems use plants that act as a source of biologically active chemical substances and can lead to the development of novel drugs. In addition to the cultural and spiritual importance of traditional medicines, they are used by about 80% of the population in developing countries, and provide an alternative to costly Western...
Inflammation, a response to infection or injury, is characterized by pain, heat, swelling, redness and disrupted physiological functions. Chemical mediators that are released from injured tissue and migrating cells can participate in and induce inflammation[3]. Increased blood flow produces heat and redness, increased vascular permeability with swelling and activation and sensitization of primary afferent nerve fibres that result in pain[4]. Non-steroidal anti-inflammatory drugs (NSAIDs) are generally used for the management of inflammatory conditions, however, various adverse effects are common, especially gastric ulcers[3]. It is evident that drugs derived from natural products are able to affect a wide range of pathways involved in the inflammatory response, including various inflammatory mediators (cytokines, arachidonic acid metabolites, excitatory amino acids, peptides, etc.); the expression of key pro-inflammatory molecules such as cyclooxygenase-2 (COX-2), neuropeptides, inducible nitric oxide synthase (iNOS), proteases, cytokines interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α); the production and/or action of secondary messengers (protein kinases, cyclic guanosine monophosphate (cGMP), cyclic adenosine monophosphate (cAMP) and calcium); the expression of transcription factors such as nuclear factor-κB (NF-κB), activator protein-1 (AP-1), and proto-oncogenes (c-fos, c-myc and c-jun)[1].

Many diseases include pain as a common and distressing symptom. Analgesics act on the central nervous system or on peripheral pain without significantly changing consciousness[3]. The pain sensation is reduced by analgesics by reducing the sensitivity of pain sensing systems to external stimuli[6].

Elevation of body temperature, called pyrexia or fever, can be initiated by inflammation, graft rejection, malignancy or tissue damage. Pyrexia may produce large amounts of IL, TNF-α, interferon and cytokines, which enhance prostaglandin E2 (PGE_2) that, in turn, activates the hypothalamus to raise body temperature. Symptoms of fever include depression, inability to concentrate, lethargy, anorexia and sleepiness. These can be accompanied by increased muscle contraction and shivering[6]. However, COX-2 expression is inhibited by most of the antipyretic drugs by hindering PGE_2 biosynthesis, and interrupting the feedback loop, leading to the reduction of the fever. These synthetic agents express high selectivity and irreversible inhibition of COX-2, however many of these drugs can have toxic effects on the glomeruli, heart muscles, cortex of brain and hepatic cells. Problems of toxicity can be reduced by substituting COX-2 inhibitors from natural sources, which have lower selectivity, for the synthetic COX-2 inhibitors[7].

*Microcos paniculata* L. of Tiliaceae family is locally known as ‘Kathgua’ or ‘Fattashi’ in Bangladesh. It has the growth form of a shrub or small tree, and grows wild and in cultivation throughout Bangladesh. Traditionally the plant is used to treat fever, diarrhea, dyspepsia, heat stroke, colds, hepatitis and wounds, for its activity in the digestive system and to kill insects. A review of the literature showed that *M. paniculata* has been found to have a wide range of activities, including larvicidal, insecticidal, free-radical-scavenging, antimicrobial, brine shrimp lethality, antiinflammatory, analgesic, α-glucosidase inhibition, cytotoxic and nicotinic receptor antagonistic activities, as well as preventative effects for coronary heart disease and angina pectoris[8–14].

The present experiments were designed to identify phytoconstituents contained in, and to evaluate the anti-inflammatory, analgesic and antipyretic activities of the methanolic extracts of bark (BME) and fruit (FME) of *M. paniculata*.

## 2 Materials and methods

### 2.1 Plant materials: collection and identification

*M. paniculata* barks and fruits were collected from the Jahangimagar University campus, Savar, Dhaka, Bangladesh in November, 2013. Species identification was verified by Sarder Nasir Uddin, Principal Scientific Officer at the Bangladesh National Herbarium (accession number 35348). A dried specimen was deposited in the herbarium for future reference.

### 2.2 Extraction

Methanol extractions were performed separately on 200 g of powdered bark or fruit of *M. paniculata*. Fresh bark and fruits were rinsed 3–4 times successively with running water and once with sterile distilled water. Washed plant materials were then dried in the shade for a period of 7 d. The dried plant materials were then ground by using a laboratory grinding mill (Model 2000 LAB Eriez®) and passed through a 40-mesh sieve to get fine powders. These bark and fruit powders (200 g) were separately extracted in 2 L of methanol each, using a soxhlet apparatus and a hot extraction procedure. Whatman No.1 filter papers were used to filter the liquid extracts. The filtrates were then dried in a hot air oven at 40 °C. The extraction yields of BME and FME were 2.96% (w/w) and 11.08% (w/w) respectively. Extracts were stored at 4 °C for additional studies.

### 2.3 Phytochemical screening

Freshly prepared *M. paniculata* plant extracts were subjected to different qualitative tests.

#### 2.3.1 Test for carbohydrates

**2.3.1.1 Molisch’s test for carbohydrates**

Approximately 500 mg of crude extracts were dissolved in 5 mL of distilled water each and later filtered. A few drops of Molisch’s reagent (α-naphthol 10% (w/v) in 90%
Wagner’s and Dragendorff’s reagents were used to test the presence of alkaloids. Crude extracts (2 mg) were dissolved individually in 1 mL of distilled water and filtered. Next, 1 mL mixture of Fehling’s solutions A and B (a ratio of 1:1) was added to the filtrates, which were heated in a water bath for a few minutes. Formation of brick-red precipitate confirmed the presence of alkaloids.

2.3.2 Tests for alkaloids

Aqueous HCl (5 mL, 1% v/v) was used to dissolve 50 mg of each extract separately and later filtered, and the filtrates were divided into 2 mL aliquots. Subsequently, Mayer’s, Wagner’s and Dragendorff’s reagents were used to test the filtrates for the presence of alkaloids.

2.3.2.1 Mayer’s test

One or two drops of 0.35 mol/L Mayer’s reagent (potassium-mercuric iodide solution, 1.36 g mercuric chloride and 5 g of potassium iodide, dissolved in 100 mL distilled H2O) were added to 2 mL of each filtrate along the side of the test tube. A positive test, demonstrating the presence of alkaloids, was indicated by a white creamy precipitate.

2.3.2.2 Wagner’s test

A few drops of 0.44 mol/L Wagner’s reagent (solution of iodine in potassium iodide, 2 g of iodine and 6 g of potassium iodide were dissolved in 100 mL distilled water) were added to 2 mL of each filtrate along the side of the test tube; a positive test, demonstrating the presence of alkaloids, was indicated by the formation of reddish-brown precipitate.

2.3.2.3 Dragendorff’s test

Dragendorff’s reagent was made of two solutions. Solution A contained 1.7 g basic bismuth nitrate in 100 mL water/glacial acetic acid (80 mL water and 20 mL glacial acetic acid in a 4:1 ratio), and solution B contained 40.0 g potassium iodide in 100 mL of water. Both solutions were mixed in following manner to produce 100 mL Dragendorff’s reagent (5 mL solution A + 5 mL solution B + 20 mL glacial acetic acid + 70 mL water). Dragendorff’s reagent at 0.136 mol/L was added (1–2 mL) to each of the 2 mL of filtrate solutions. The formation of an orange-red precipitate indicated the presence of alkaloids.

2.3.3 Frothing test for saponin

Crude extracts (100 mg) of fruit and bark were each dissolved in 10 mL of methanol for making stock solutions. These stock solutions were diluted to 0.5 mg/mL by the additions of 20 mL of distilled water. Test tubes containing the dilution were then shaken for 15 min. Formation of foam on the top of the test tubes indicated the presence of saponin.

2.3.4 FeCl3 test for tannins

Crude extracts (50 mg) were dissolved in 5 mL distilled water, followed by the addition of a few drops of 5% FeCl3. Tannin was confirmed by the development of a bluish-black color.

2.3.5 Alkali test for flavonoids

A few drops of 5% NaOH solution were added to 1 mL of filtered stock solutions (100 mg of respective extract/10 mL of methanol), which produced a deep-yellow color. The color was lost in the presence of dilute HCl and confirmed flavonoids.

2.3.6 Salkowski’s test for triterpenoids

Plant extracts (2 mg) were separately shaken in 1 mL of CHCl3. A few drops of concentrated H2SO4 were added to these solutions along the side of the test tube. Development of a red-brown color at the interface indicated the presence of triterpenoids.

2.3.7 Baljet test for glycosides

Each filtered stock solutions (1 mL) of the plant extracts were individually added to 1 mL of sodium picrate solution. And the sodium picrate solution was immediately made before use by the addition of 95 mL 1% (w/v) aqueous picric acid with 5 mL 10% (w/v) aqueous NaOH and filtered through Whatman No.1 filter paper. That filtrate was used as Baljet reagent. The transformation of the sodium picrate solution’s yellow color to orange confirmed the presence of cardiac glycosides.

2.4 Experimental animals

Two hundred and ten Swiss albino mice of either sex, 6–7 weeks old, weighting 25–30 g (Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh) were used in these experiments. These animals were kept under standard environmental conditions, having relative humidity 55%–65%, 12 h light/12 h dark cycle and (27±1°C) temperature. Proper supply of foods and water ad libitum were ensured. Before the experiment, animals were adapted to the laboratory conditions for 1 week. The Institutional Animal Ethical Committee of Jahangirnagar University, Savar, Dhaka, Bangladesh approved all the protocols used in the experiments conducted with these animals.

2.5 Acute toxicity study

Adverse effects that result either from a single exposure or from multiple exposures over a short time (normally less than 24 h) are known as acute toxicity. To find the half lethal dose (LD50) of the experimental samples, the acute toxicity study was carried out following the Organization of Economic Cooperation and Development (OECD) guidelines. Fifteen mice were divided into three groups: control group and test groups (BME or FME), with five animals per group. The experimental samples (BME or FME) were administered orally at different concentrations (100, 250, 500, 1 000, 2 000, 3 000 and 4 000 mg/kg body weight). After that the animals were observed every 1 h.
for next 5–6 h for mortality, behavioral pattern changes such as weakness, aggressiveness, food or water refusal, diarrhea, salivation, discharge from eyes and ears, noisy breathing, changes in locomotor activity, convulsion, coma, injury, pain or any signs of toxicity in each group of animals. A final evaluation at the end of a 2-week observation period was also conducted.[22]

2.6 In-vitro anti-inflammatory study
The in-vitro anti-inflammatory study was conducted by detecting proteinase inhibitory activity following the methods of Oyedapo et al.[23] and Kumarappan et al.[24]. Briefly, trypsin (0.06 mg), 25 mmol/L tris-HCl buffer (1 mL, pH 7.4) and 1.0 mL aqueous solution of aspirin (standard drug) or the various extracts of M. paniculata (50–250 µg/mL) were combined to make 2 mL reaction volumes. After 5 min of incubation at 37 °C, 1 mL of 0.8% casein (w/v) was added and incubation was continued for 20 additional minutes. The reaction was terminated by the addition of 2.0 mL 70% perchloric acid. A cloudy suspension formed and was centrifuged later at 1109 × g for 10 min. The absorbance of the supernatant was measured at 280 nm, using the reaction buffer as a blank. The percentage of proteinase inhibition and half inhibitory concentration (IC50) values were calculated taking the average of triplicate values.

2.7 In-vivo anti-inflammatory study
2.7.1 Xylene-induced ear edema
The xylene-induced ear edema test was performed according to Dai et al.[25]. Thirty mice were divided into control group (normal water), positive control or standard group (diclofenac sodium, DS, 100 mg/kg body weight), and test groups (BME & FME at 200 and 400 mg/kg body weight), containing five mice in each group. Mice in the control group, positive control group and test groups received one dose of normal water, diclofenac sodium, methanolic extracts of barks & fruits orally. One hour after treatment, each animal received 20 µL of xylene on the anterior and posterior surfaces of the right ear lobe. The left ear was untreated, and used as a control. Mice were sacrificed 1 h after xylene application and 3 mm circular sections of the ears were taken and weighed. The weight of xylene-induced edema was considered as the difference between weight of ear treated with xylene (right ear) and the weight of ear without xylene treatment (left ear).

The percentage inhibition of ear edema was calculated by the following formula:

\[
\text{Inhibition} (%) = \left[1 - \frac{\text{Weight of edema (extract or standard drug)}}{\text{Weight of edema (normal control)}}\right] \times 100
\]

2.7.2 Cotton pellet-induced granuloma formation
The method of Swingle and Shideman[26] was used to evaluate the granuloma formation in mice. Sterilized cotton pellets, weighing (10±1) mg each, were inserted subcutaneously, one on each side of the abdomen of the animal, under light chloroform anesthesia and sterile technique. Mice of each group received treatment doses orally, as described above, once a day for 7 d. The mice were sacrificed on the 8th day. Cotton pellets were removed and dried at 60 °C for 24 h. The dry cotton weight was recorded. The weight difference between the removed, dried cotton pellets, and the cotton pellets before insertion was considered to be the weight of granuloma formed.

The percentage inhibition of granuloma formation was calculated by the following formula:

\[
\text{Inhibition} (%) = \left[1 - \frac{\text{Weight of granuloma (extract or standard drug)}}{\text{Weight of granuloma (normal control)}}\right] \times 100
\]

2.8 Analgesic study
2.8.1 Formalin-induced paw licking
The method of Hunskaar and Hole[27] was used for the paw licking study. Mice grouping and administration were performed as mentioned before. After 1 h of treatment of each group, 2.7% formalin was injected into the dorsal surface of the left hind paw of each mouse. The time spent for licking the injected paw was recorded. Animals were observed for 5 min after formalin injection (acute phase) and for 5 min in delayed phase, which was starting at the 20th minute after formalin injection.

The percentage inhibition of licking was calculated using the following formula:

\[
\text{Inhibition} (%) = \left[1 - \frac{\text{Licking time (extract or standard drug)}}{\text{Licking time (normal control)}}\right] \times 100
\]
2.8.2 Acetic acid-induced writhing test
The method of Koster et al.\textsuperscript{28} was employed for the writhing test. Mice were pretreated with extracts as mentioned before. DS (100 mg/kg) was used as standard or positive control and water as normal control. Forty-five minutes later, each mouse was injected intraperitoneally with 0.7% acetic acid at a dose of 10 mL/kg body weight. Fifteen minutes after administration of acetic acid, the number of writhing responses was recorded for each animal during a 5-minute period, the mean abdominal writhing for each group was calculated.

The percentage inhibition of writhing was calculated using the following formula:

\[
\text{Inhibition} (\%) = \left[ 1 - \frac{\text{No. of writhing (extract or standard drug)}}{\text{No. of writhing (normal control)}} \right] \times 100
\]

2.8.3 Tail immersion test
The method of Toma et al.\textsuperscript{29} was employed for this test. The method was used to evaluate the central mechanism of analgesic activity. Here the painful reactions in animals were generated by thermal stimulus through dipping the tip of the tail in hot water. Mice were grouped and treated as described before. Tramadol (10 mg/kg) was used as the reference drug. The animals were fasted for 16 h with water ad libitum. After the treatment of each group, the basal reaction time was measured by immersing the tail tips of the mice (last 1–2 cm) in hot water of (55 ± 1) °C. The flick response of mice, i.e., time taken (in second) to withdraw it from hot water source was calculated and results were compared with the control group. A latency period of 15 s was set as the cut-off point to avoid injury to mice. The latent period of the tail-flick response was determined before 30 min and after 30, 60, 120 and 180 min of the respective treatment of each group.

2.9 Brewer’s yeast-induced pyrexia test
This test was performed with slight modification as described by Turner et al.\textsuperscript{30}. Mice were fasted overnight with water ad libitum before inducing pyrexia. Their rectal temperatures were recorded before inducing pyrexia by using an electric thermometer, which was connected with a probe and inserted 2 cm into the rectum. Subcutaneous injection of a 15% (w/v) suspension of brewer’s yeast at a dose of 10 mL/kg in the back below the nape of the neck induced pyrexia. The suspension was spread under the skin by massaging the injection site. The increase in rectal temperature was recorded 18 h after injection, and the mice that showed an increase in temperature of at least 0.6 °C were considered pyretic mice and used for brewer’s yeast-induced pyrexia test. The tested samples including paracetamol (100 mg/kg) as standard, normal water as control and plant extracts as described before, which were given orally to the pyretic mice, were investigated for their antipyretic activity. All group’s temperatures were recorded at 1, 2, 3 and 4 h.

2.10 Statistical analysis
All results are expressed as mean ± standard error of mean (SEM). Statistical analysis for % proteinase-inhibitory activity was evaluated by one-way analysis of variance (ANOVA) followed by post-hoc Tukey’s HSD test for pair-wise comparison of means among the groups. IC\textsubscript{50} values were calculated by linear regression equations (Microsoft Excel 2007; Microsoft, Redmond, Washington, USA) and the statistical significance among the groups was shown through post-hoc Bonferroni test. Without the in-vitro anti-inflammatory test, all other tests were analyzed statistically by one-way ANOVA followed by Dunnett’s t test. In addition, the results of tail immersion test & brewer’s yeast-induced pyrexia test were analyzed by using repeated measure ANOVA (RM-ANOVA). In case of all in-vivo studies, pair-wise comparison of means among the groups was done by one-way ANOVA followed by post-hoc Tukey’s HSD test. P<0.05 was considered to be statistically significant. All data were analyzed using SPSS software (version 17; IBM Corporation, New York, USA).

3 Results
3.1 Phytochemical screening
During the evaluation of candidate plant materials for pharmacological activities, the characterization of their chemical nature is essential. Phytochemical screening of the BME and FME showed the presence of several primary and secondary metabolites, or phytoconstituents, which are summarized in Table 1. In the phytochemical screening, BME and FME were shown to have different compositions. BME showed the presence of almost all of the phytoconstituents like carbohydrates, alkaloids, saponins, tannins, flavonoids and triterpenoids that were tested here. However, some tests did not show consistent results. Carbohydrate content in FME was indicated by Molisch’s test, but not by Fehling’s test. Similarly, Wagner’s test indicated that alkaloids were not present in FME, but they were found by the Mayer’s and Dragendorff’s tests. Glycosides were absent in both BME and FME.

3.2 Acute toxicity study
No mortality, or signs of toxicity or behavioral changes were observed during the 14-day observation period in mice receiving doses up to 4 000 mg/kg of BME or FME (test groups). The control group showed the same result. This
demonstrates that the test groups do not experience acute oral toxicity at the doses tested.

3.3 In-vitro anti-inflammatory study

In the present study, the plant extracts were found to possess significant antiproteinase activity (Table 2). The highest anti-proteinase activity measured for *M. paniculata* extracts was 75.94% in BME at a dose of 250 μg/mL. Aspirin had the lowest IC50, followed by BME and FME (Table 3).

3.4 In-vivo anti-inflammatory study

3.4.1 Xylene-induced ear edema in mice

Anti-inflammatory activities of BME and FME on topical xylene-induced ear edema in mice are shown in Table 4. The application of xylene to the mouse ear rapidly induced cutaneous inflammation. All of the groups had significant ear weight differences and inhibition of ear edema when compared to the control. Among the extracts, highest percentage inhibition of ear edema (36.97%) was observed by BME at 400 mg/kg.

3.4.2 Cotton pellet-induced granuloma formation in mice

The results of the chronic inflammatory test with cotton pellet are displayed in Table 5. All of the groups had significant granuloma weight and percentage inhibition of granuloma formation in mice when compared to the control. Among the plant extracts, BME at a dose of 400 mg/kg showed highest percentage inhibition (45.96%) of granuloma formation in mice.

3.5 Analgesic study

3.5.1 Formalin-induced paw licking in mice

Of the plant extracts, BME at 400 mg/kg body weight showed significant highest percentage inhibition (78.01%) of paw licking in mice during the late phase of formalin injection when compared to the control. In addition, DS was effective at both acute and delayed phase (Table 6). Again, BME at 200 and 400 mg/kg revealed a little increase of percentage inhibition of paw licking from acute phase to delayed phase. But, percentage inhibition of paw licking decreased in late phase for FME at 200 and 400 mg/kg.

3.5.2 Acetic acid-induced writhing test

In the mouse writhing assay, all groups caused significant percentage inhibition of writhing when compared to the control (Table 7). Treatment with DS (100 mg/kg) resulted in less writhing than treatment with either of the extracts and either of the doses. The maximum percentage inhibition of writhing resulting from treatment with plant extracts (53.82%) was obtained by FME at 400 mg/kg.

### Table 1 Phytochemical screening of BME and FME of *M. paniculata*

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Test name</th>
<th>Observation of various extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>Molisch’s test</td>
<td>BME + FME +</td>
</tr>
<tr>
<td></td>
<td>Fehling’s test</td>
<td>BME + FME -</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Mayer’s test</td>
<td>BME + FME +</td>
</tr>
<tr>
<td></td>
<td>Wagner’s test</td>
<td>BME + FME -</td>
</tr>
<tr>
<td></td>
<td>Dragendorff’s test</td>
<td>BME + FME +</td>
</tr>
<tr>
<td>Saponins</td>
<td>Frothing test</td>
<td>BME + FME +</td>
</tr>
<tr>
<td>Tannins</td>
<td>FeCl₃ test</td>
<td>BME + FME -</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Alkali test</td>
<td>BME + FME -</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>Salkowski’s test</td>
<td>BME + FME +</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Baljet test</td>
<td>BME - FME -</td>
</tr>
</tbody>
</table>

+: presence of specific phytoconstituents; -: absence of specific phytoconstituents.

### Table 2 Proteinase-inhibitory activity of the standard drug and different extracts of *M. paniculata*

<table>
<thead>
<tr>
<th>Test samples</th>
<th>n</th>
<th>50 µg/mL</th>
<th>100 µg/mL</th>
<th>150 µg/mL</th>
<th>200 µg/mL</th>
<th>250 µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>3</td>
<td>52.86±0.42</td>
<td>67.86±0.45</td>
<td>81.17±0.52</td>
<td>88.95±0.08</td>
<td>97.33±0.17</td>
</tr>
<tr>
<td>BME</td>
<td>3</td>
<td>44.24±0.33 △</td>
<td>58.45±0.25 △</td>
<td>67.68±0.21 △</td>
<td>71.43±0.22 △</td>
<td>75.94±0.26 △</td>
</tr>
<tr>
<td>FME</td>
<td>3</td>
<td>4.41±0.06 △▲</td>
<td>13.88±0.56 △▲</td>
<td>50.13±0.09 △▲</td>
<td>52.46±0.27 △▲</td>
<td>56.23±0.12 △▲</td>
</tr>
</tbody>
</table>

Values are present as mean ± standard error of mean. Tukey HSD test was performed for the pair-wise mean comparison. △ P < 0.05, vs aspirin group; ▲ P < 0.05, vs BME group. BME: extract of *Microcos paniculata* bark; FME: extract of *Microcos paniculata* fruit.

### Table 3 IC5₀ of the standard and different extracts of *M. paniculata*

<table>
<thead>
<tr>
<th>Test samples</th>
<th>n</th>
<th>IC5₀ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>3</td>
<td>24.46±1.80</td>
</tr>
<tr>
<td>BME</td>
<td>3</td>
<td>61.31±1.81 △</td>
</tr>
<tr>
<td>FME</td>
<td>3</td>
<td>201.55±0.61 △▲</td>
</tr>
</tbody>
</table>

Values are present as mean ± standard error of mean. Bonferroni test was performed for the pair-wise mean comparison. △ P < 0.05, vs aspirin group; ▲ P < 0.05, vs BME group. BME: extract of *Microcos paniculata* bark; FME: extract of *Microcos paniculata* fruit.
Table 4  Effects of the standard and different extracts of M. paniculata in xylene-induced ear edema test

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>n</th>
<th>Ear weight differences (mg)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 mL/kg</td>
<td>5</td>
<td>13.10±0.30</td>
<td></td>
</tr>
<tr>
<td>DS</td>
<td>100 mg/kg</td>
<td>5</td>
<td>7.42±0.12*</td>
<td>43.24±1.49*</td>
</tr>
<tr>
<td>BME</td>
<td>200 mg/kg</td>
<td>5</td>
<td>10.54±0.05* □</td>
<td>19.34±2.19*</td>
</tr>
<tr>
<td>BME</td>
<td>400 mg/kg</td>
<td>5</td>
<td>8.24±0.68*</td>
<td>36.97±5.45*</td>
</tr>
<tr>
<td>FME</td>
<td>200 mg/kg</td>
<td>5</td>
<td>10.02±1.15* □</td>
<td>22.73±10.57*</td>
</tr>
<tr>
<td>FME</td>
<td>400 mg/kg</td>
<td>5</td>
<td>8.42±0.08*</td>
<td>35.59±1.56*</td>
</tr>
</tbody>
</table>

Values of ear weight differences are present as mean ± standard error of mean. *P<0.05, vs control (Dunnett’s t test); □ P < 0.05, vs DS 100 mg/kg group (pair-wise comparison by post-hoc Tukey’s HSD test). DS: diclofenac sodium; BME: extract of Microcos paniculata bark; FME: extract of Microcos paniculata fruit.

Table 5  Effects of the standard and different extracts of M. paniculata in cotton pellet induced granuloma test

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>n</th>
<th>Granuloma weight (mg/mg cotton)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 mL/kg</td>
<td>5</td>
<td>22.60±0.35</td>
<td></td>
</tr>
<tr>
<td>DS</td>
<td>100 mg/kg</td>
<td>5</td>
<td>11.24±0.16*</td>
<td>50.24±0.73*</td>
</tr>
<tr>
<td>BME</td>
<td>200 mg/kg</td>
<td>5</td>
<td>15.63±0.11* □</td>
<td>30.78±0.77*</td>
</tr>
<tr>
<td>BME</td>
<td>400 mg/kg</td>
<td>5</td>
<td>12.20±0.07* □□</td>
<td>45.96±0.90*</td>
</tr>
<tr>
<td>FME</td>
<td>200 mg/kg</td>
<td>5</td>
<td>15.90±0.05* □□☆</td>
<td>29.76±0.98*</td>
</tr>
<tr>
<td>FME</td>
<td>400 mg/kg</td>
<td>5</td>
<td>13.13±0.06* □□☆□★</td>
<td>41.86±0.99*</td>
</tr>
</tbody>
</table>

Weights of cotton pellets are present as mean ± standard error of mean. *P<0.05, vs control (Dunnett’s t test); □ P < 0.05, vs DS 100 group; ■ P < 0.05, vs BME 200 group; ☆ P < 0.05, vs BME 400 group; ★ P < 0.05, vs FME 200 group (pair-wise comparison by post-hoc Tukey’s HSD test). DS: diclofenac sodium; BME: extract of Microcos paniculata bark; FME: extract of Microcos paniculata fruit.

Table 6  Effects of various extracts of M. paniculata in formalin-induced licking test

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>n</th>
<th>Acute phase</th>
<th>Licking time (s)</th>
<th>Inhibition (%)</th>
<th>Delayed phase</th>
<th>Licking time (s)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 mL/kg</td>
<td>5</td>
<td></td>
<td>224.47±2.28</td>
<td></td>
<td></td>
<td>75.80±1.39</td>
<td></td>
</tr>
<tr>
<td>DS</td>
<td>100 mg/kg</td>
<td>5</td>
<td></td>
<td>144.20±1.98*</td>
<td>35.76±0.48*</td>
<td></td>
<td>6.00±1.05*</td>
<td>92.03±1.41*</td>
</tr>
<tr>
<td>BME</td>
<td>200 mg/kg</td>
<td>5</td>
<td></td>
<td>122.52±1.56* □</td>
<td>45.42±0.44*</td>
<td></td>
<td>39.20±3.54* □</td>
<td>48.34±4.31*</td>
</tr>
<tr>
<td>BME</td>
<td>400 mg/kg</td>
<td>5</td>
<td></td>
<td>64.80±1.24* □☆</td>
<td>71.13±0.45*</td>
<td></td>
<td>16.60±1.44* □☆</td>
<td>78.01±2.10*</td>
</tr>
<tr>
<td>FME</td>
<td>200 mg/kg</td>
<td>5</td>
<td></td>
<td>102.75±2.46* □☆</td>
<td>54.01±0.43*</td>
<td></td>
<td>53.40±2.34* □☆</td>
<td>29.41±3.46*</td>
</tr>
<tr>
<td>FME</td>
<td>400 mg/kg</td>
<td>5</td>
<td></td>
<td>75.20±1.56* □☆☆</td>
<td>66.51±0.50*</td>
<td></td>
<td>50.20±1.07* □☆☆</td>
<td>33.67±1.93*</td>
</tr>
</tbody>
</table>

Values of the first and second 5 min are present as mean ± standard error of mean. *P<0.05, vs control (Dunnett’s t test); □ P < 0.05, vs DS 100 group; □ P < 0.05, vs BME 200 group; □ P < 0.05, vs BME 400 group; □ P < 0.05, vs FME 200 group (pair-wise comparison by post-hoc Tukey’s HSD test). DS: diclofenac sodium; BME: extract of Microcos paniculata bark; FME: extract of Microcos paniculata fruit.

Standard drug, DS, at 100 mg/kg body weight, showed 80.06% writhing inhibition.

3.5.3 Tail immersion test
At 30 min after administration, only the tramadol group had a significant increase in latency (Table 8). By 60 min, the effects of BME at 400 mg/kg, and FME at 200 and 400 mg/kg had significant analgesic activities relative to the control. The maximum effects of the extracts were obtained at 60 min. But BME 200 mg/kg did not demonstrate any significant increase in latency at any time point. It was observed that tramadol showed significant analgesic effects at 30, 60, 120 and 180 min (P<0.05 vs control at each of the cases).

3.6 Antipyretic study
Subcutaneous injection of yeast caused the elevation of rectal temperature after 18 h of its administration.

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The experimental mice showed a mean increase of about −16.64 °C rectal temperature 18 h after brewer’s yeast injection. The antipyretic effects of the different doses of the extracts (200 and 400 mg/kg), standard (paracetamol, 100 mg/kg), and control are shown in Table 9. Most of the significant antipyretic activities of the test samples were found after the 1st or 2nd hour of their administration. BME (200 and 400 mg/kg) and FME (400 mg/kg) showed significant post-treatment antipyretic activities, but FME 200 mg/kg did not show any significant post-treatment antipyretic activity when compared to control.

4 Discussion

Phytochemical components are identified as bioactive compounds of plant extracts and may be responsible for the diverse activities when herbs are used medicinally[31]. Secondary herbal metabolites have influence on the medicinal and pharmacological actions of medicinal herbs. Primary metabolites (e.g., amino acids, monosaccharides, nucleic acids, polysaccharides, proteins, lipids) are present in almost all plant species, whereas secondary metabolites are found in fewer plant species; in the plant, these compounds provide defences against herbivores and pathogens, attract pollinators and fruit dispersers, give mechanical support, and pharmacological actions of medicinal herbs. Primary herbal metabolites have influence on the medicinal

Table 7 Effects of different extracts of M. paniculata in acetic acid-induced writhing test

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>n</th>
<th>Number of writhing</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 mL/kg</td>
<td>5</td>
<td>11.80±0.37</td>
<td>−</td>
</tr>
<tr>
<td>DS</td>
<td>100 mg/kg</td>
<td>5</td>
<td>2.40±1.03</td>
<td>80.06±8.48*</td>
</tr>
<tr>
<td>BME</td>
<td>200 mg/kg</td>
<td>5</td>
<td>8.80±0.66</td>
<td>25.50±4.59*</td>
</tr>
<tr>
<td>BME</td>
<td>400 mg/kg</td>
<td>5</td>
<td>6.80±0.37</td>
<td>42.39±2.45*</td>
</tr>
<tr>
<td>FME</td>
<td>200 mg/kg</td>
<td>5</td>
<td>6.60±0.24</td>
<td>43.78±3.11*</td>
</tr>
<tr>
<td>FME</td>
<td>400 mg/kg</td>
<td>5</td>
<td>5.40±0.24</td>
<td>53.82±3.47*</td>
</tr>
</tbody>
</table>

Number of writhing is present as mean ± standard error of mean. □ *P < 0.05, vs control (Dunnett’s t test); □ ■ *P < 0.05, vs DS 100 group; □ □ ■ *P < 0.05, vs BME 200 group (pair-wise comparison by post-hoc Tukey’s HSD test).

Table 8 Effects of various extracts of M. paniculata on latency time in tail immersion test

<table>
<thead>
<tr>
<th>Group</th>
<th>Doses</th>
<th>n</th>
<th>Latency time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Control</td>
<td>10 mL/kg</td>
<td>5</td>
<td>1.74±0.25</td>
</tr>
<tr>
<td>Tramadol</td>
<td>100 mg/kg</td>
<td>5</td>
<td>2.40±0.16</td>
</tr>
<tr>
<td>BME</td>
<td>200 mg/kg</td>
<td>5</td>
<td>2.40±0.30</td>
</tr>
<tr>
<td>BME</td>
<td>400 mg/kg</td>
<td>5</td>
<td>2.20±0.27</td>
</tr>
<tr>
<td>FME</td>
<td>200 mg/kg</td>
<td>5</td>
<td>2.20±0.07</td>
</tr>
<tr>
<td>FME</td>
<td>400 mg/kg</td>
<td>5</td>
<td>2.20±0.14</td>
</tr>
</tbody>
</table>

Latency time values are present as mean ± standard error of mean. 0 min means 30 min before drug administration, +30 min, +60 min, +120 min, and +180 min indicate 30, 60, 120, and 180 min after drug administration, respectively. Tests of within-subjects effects reveal that for the factor ‘Time’ calculated F = 34.161 for all methods and P value = 0.000 in every case. So time is highly significant at any level of significance. □ P < 0.05, vs control. Repeated measure analysis of variance with Dunnett’s multiple comparison was performed to analyze this data set. BME: extract of Microcos paniculata bark; FME: extract of Microcos paniculata fruit.

Table 9 Antipyretic effects of various extracts of M. paniculata

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>n</th>
<th>Initial rectal temperature</th>
<th>Rectal temperature after 18 h of yeast injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0 h</td>
<td>1 h</td>
</tr>
<tr>
<td>Control</td>
<td>10 mL/kg</td>
<td>5</td>
<td>32.17±0.22</td>
<td>32.94±0.23</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>100 mg/kg</td>
<td>5</td>
<td>31.14±0.55</td>
<td>32.71±0.37</td>
</tr>
<tr>
<td>BME</td>
<td>200 mg/kg</td>
<td>5</td>
<td>31.47±0.29</td>
<td>33.07±0.26</td>
</tr>
<tr>
<td>BME</td>
<td>400 mg/kg</td>
<td>5</td>
<td>31.36±0.16</td>
<td>32.34±0.14</td>
</tr>
<tr>
<td>FME</td>
<td>200 mg/kg</td>
<td>5</td>
<td>30.88±0.26*</td>
<td>32.03±0.07*</td>
</tr>
<tr>
<td>FME</td>
<td>400 mg/kg</td>
<td>5</td>
<td>31.64±0.14</td>
<td>32.39±0.13</td>
</tr>
</tbody>
</table>

Rectal temperature values (°C) are present as mean ± standard error of mean. Tests of within-subjects effects reveal that for the factor ‘Time’ calculated F = 50.844 for all methods and P value = 0.000 in every case. So time is highly significant at any level of significance. □ P < 0.05, vs control. Repeated measure analysis of variance with Dunnett’s multiple comparison was performed to analyze this data set. BME: extract of Microcos paniculata bark; FME: extract of Microcos paniculata fruit.
absorb harmful ultraviolet radiation and reduce the growth of nearby competing plants. Alkaloids, phenolics (simple phenolics and flavonoids), terpenoids, fatty acids, glycosides, waxes and their derivatives are examples of secondary metabolites that can have medicinal properties. In the field of drug discovery and development, the preliminary screening of secondary metabolites facilitates the recognition of bioactive compounds.

Although many plant-derived products are in use in systems of traditional medicine, scientifically rigorous toxicity studies have been conducted on very few. Hence, acute oral toxicity studies are extremely important to determine the proper range of doses for subsequent usage and to identify the potential adverse effects of the materials under examination. During the investigation of therapeutic index of drugs and xenobiotics, acute oral toxicity study becomes a suitable factor.

LD₅₀ of the plant extracts could not be obtained, as no mortality was observed up to the dose as high as 4000 mg/kg and the extracts were found to be safe with a broad therapeutic range. Therefore, two comparatively high doses (200 and 400 mg/kg) for both FME and BME were used for in-vivo doses.

In the inflammatory response, the expansion of tissue damage is mediated by proteinases. Thus, the ability to inhibit proteinase activity is representative of the level of protection against damage caused by inflammation. Trypsin catalyzes the hydrolysis of peptide bonds, which are formed from the carboxyl groups of basic amino acids, and is found in the pancreatic juice as an endoprotease enzyme. Proteinase-activated receptor-2 (PAR2) is activated by trypsin. Inflammatory responses are stimulated by trypsin and PAR2 through the production of cytokines, such as IL-6, IL-8, PG and via the p65-NF-κB pathway. Proinflammatory effects including leukocyte-endothelial interactions, vasodilatation, reflex esphagitis and edema are produced by PAR-2 activation. The anti-inflammatory activities of many plants are linked to the presence of flavonoids and related polyphenols in their tissues. Therefore, the existence of alkaloids, flavonoids, tannins, triterpenoids and saponins in BME and alkaloids, triterpenoids and saponins in FME may be responsible for their significant anti-inflammatory effect.

Anti-inflammatory topical steroids or non-steroidal anti-inflammatory agents, which inhibit phospholipase A2, can be evaluated by the xylene-induced ear edema test. It is an acute inflammation test and can release inflammatory mediators such as bradykinin, histamine and serotonin, which stimulate ear edema by increasing vascular permeability and promoting vasodilation. The xylene-induced ear edema test shows fluid accumulation at the treatment site; inhibiting this fluid accumulation is regarded as an anti-inflammatory effect. The significant inhibition of ear edema by BME and FME may be due to the hindrance of phospholipase A2 and by decreasing vascular permeability and vasodilation. But further study is required to confirm the mechanism by which the plant extracts inhibited edema.

Proliferation of inflammatory cells like neutrophils, macrophages and fibroblasts is responsible for granuloma formation in the cotton pellet-induced granuloma model. It is used as an in vivo chronic inflammatory test. Subcutaneous implantation of cotton pellet in mice leads to at least three phases of response, including transudative phase, exudative phase, and proliferative phase. The host inflammatory response and the modulation of inflammatory mediators are induced by the implanted material, which finally leads to tissue proliferation and granular formation. Steroidal anti-inflammatory drugs have a strong inhibition on both the proliferative and transudative phases of inflammation, whereas NSAIDs, such as diclofenac sodium, provide inhibition in only the late phase, by inhibiting PG synthesis. Diclofenac sodium and the plant extracts reduced the wet cotton pellet weight (Table 5), an indication of reduction in accumulation of exudates at the inflammatory site. Therefore, the decrease in the weight of granuloma by the plant extracts may be due to the suppression of proliferative phase. Analysis of additional biochemical pathways may confirm the significant reduction of granuloma weight by the plant extracts.

Peripheral and central activities of nociception are revealed by the formalin test. Two different periods of rigorous licking activity, an early response (0–5 min after the injection of formalin) and a late response (20–30 min after the injection of formalin) occur in this test. The early phase is caused by the direct effect of formalin on nociceptors (non-inflammatory pain), while the late phase reflects pain from formalin-induced inflammation. Substance P participates in the early phase, whereas serotonin, histamine, PGs, and bradykinin are involved in the late phase. Thus, the analgesic effect of the plant extracts may depend on either central or peripheral sites of action. Both phases of pain can be inhibited by centrally acting drugs, such as opioids through the equal inhibition of effects produced by PGs and by endogenous opioids through their action on the central nervous system. The late response is inhibited by peripheral analgesics only, such as acetylsalicylic acid, whereas both early and late responses are inhibited by the narcotic analgesics. BME inhibited the percentage inhibition of licking at both phases more than FME. However, extensive studies are required to explore the analgesic mechanism of the plant extracts.

In writhing response experiments, the sensation of pain arises through the activation of the localized inflammatory response by the acetic acid. Free arachidonic acid from the tissue phospholipids is discharged by the pain stimulus.
To assess peripherally acting analgesics, the acetic acid-induced writhing response is thought to be mediated by peritoneal mast cells[50], acid-sensing ion channels[51] and the PG pathways[52]. Table 7 represents the peripheral analgesic effect of different extracts of *M. paniculata* in acetic acid-induced writhing test. These effects may represent the blocking of peritoneal mast cells, acid-sensing ion channels and/or the PG pathways.

The tail immersion model is one of the important acute pain models[53]. It evaluates the centrally acting analgesics and opioid receptor agonists that are more sensitive to this test. An opioid agent’s analgesic activities are realized through spinal (δ₁, σ₂, and κ₁) and supraspinal (δ₂, σ₁, and κ₂) receptors[54]. The opioid µ receptor agonists show more sensitivity to thermal nociceptive tests like the tail immersion test[55]. Table 8 shows the effects of various extracts of *M. paniculata* on latency time in the tail immersion test. Opioids may be antiinociceptive for both early and late phases[56]. Although opioid µ receptor agonists show more sensitivity to tail immersion test, the data presented in Table 8 point out that the extracts only showed significant activity in the early phase (i.e., at 60 min after the administration of extracts) that contradicts the involvement of opioids. So, broad studies are needed to elucidate the exact pain inhibitory mechanism of actions of the plant extracts.

Fever is provoked by many exogenous substances like bacterial endotoxins and microbial infection in animal models. Exogenous pyrogen stimulates the production of proinflammatory cytokines, such as TNF-α, IL-1β, IFN-α and IL-6, which enter into the hypothalamic circulation and the liberation of local PGs is stimulated[57]. Body temperature is increased by PGE₂[58]. The methanolic extracts of *M. paniculata* barks and fruits demonstrated antipyretic activity in the yeast-induced pyrexia model. The antipyretic action of the extracts may be due to the inhibition of PG production. Flavonoids inhibit PG synthesis[59]. Alkaloids can inhibit the PG synthesis also[60]. Hence, the presence of flavonoids and alkaloids in the BME and alkaloids in the FME of *M. paniculata* may be contributory to their antipyretic activity.

**5 Conclusion**

From the existing study, it could be suggested that the methanolic extracts of *M. paniculata* barks and fruits might possess anti-inflammatory, analgesic and antipyretic activities. Nevertheless, further quantitative chemical studies are now under way to isolate and determine the structure of the active constituents. Similarly, we are pursuing biological testing of the specific compounds thought to be responsible for anti-inflammatory, analgesic and antipyretic activities presented in the bark and fruit extracts of *M. paniculata*. Again, genotoxicity study of this plant should be carried out for safety evaluation, though in the present study the plant extracts did not show any acute oral toxicity.

**6 Acknowledgements**

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**7 Conflict of interests**

The author declares that there is no conflict of interests regarding the publication of this paper.

**REFERENCES**


