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Quantitative screening of phytochemicals and pharmacological attributions of the leaves and stem barks of *Macropanax dispermus* (Araliaceae) in treating the inflammation and arthritis

Syeda Rubaiya Afrin, Mohammad Rashedul Islam, Nawreen Monir Proma, Mahabuba Khanam Shorna, Sumaiya Akbar, Mohammed Kamrul Hossain*

Department of Pharmacy, Faculty of Biological Sciences, University of Chittagong, Chittagong-4331, Bangladesh

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ABSTRACT

Introduction: Inflammation is a major cause of arthritis. Since the conventional medicines used for the treatment of this disease have many side effects, herbal remedies can be proved to be effective in this case. So, the present study was aimed at investigating the quantitative detection of phytochemicals, screening of *in vivo* anti-inflammatory and the possible anti-arthritic activities of the crude methanol extracts of a traditional medicinal plant Macropanax dispermus leaves (MDML) and stem barks (MDMS).

Methods: Quantitative screening of phytochemical constituents was analyzed by standard procedures. The *in vivo* anti-inflammatory activity was conducted on Swiss albino mice by using carrageenan and formalin-induced paw edema tests, and xylene-induced ear edema test. The possible anti-arthritic activity was done by evaluating the *in vitro* inhibition of bovine serum albumin (BSA) denaturation.

Results: The current research showed that MDML contained a considerable amount of flavonoids and alkaloids, and MDMS contained a considerable amount of phenols. MDMS (200, 400 mg/kg) was observed to be an effective and significant ($P<0.001$) peripheral anti-inflammatory agent in carrageenan and formalin-induced paw edema tests, whereas MDML (400 mg/kg) was observed to have an effective and significant ($P<0.001$) neurogenic anti-inflammatory effect in xylene-induced ear edema test as compared to negative control group. MDMS was observed to be an effective anti-arthritic agent as compared to that of negative control group. All those effects were dose and concentration-dependent.

Conclusion: The present research proved that MDML and MDMS were effective medications for the treatment of inflammation and arthritis.

Implication for health policy/practice/research/medical education:
The investigated *Macropanax dispermus* (Araliaceae) extract exhibited significant activity against inflammation and arthritis and might contribute to formulate alternative medicinal agent to treat inflammatory disorders.

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Introduction

Inflammation is a reaction of the body against noxious stimuli, characterized by vasodilatation, access of fluid and cells to the target tissue (1). It is also characterized by the increase in vascular permeability and mediator release (2), an increase of protein denaturation and membrane alteration (3). Further, leucocyte infiltration, edema and granuloma formation demonstrate common features of inflammation (4).

Arthritis is one of the most common chronic inflammatory disorders, a systemic autoimmune disease and the prime cause of disability worldwide. Arthritis causes the breakdown of cartilage that results in the friction of bones together. It causes pain, swelling and stiffness of joints of bones (5). It can be treated by using drugs such as non-steroidal anti-inflammatory drugs

***Corresponding author**: Mohammed Kamrul Hossain,
Email: mkhossain73@yahoo.com

(NSAIDs), corticosteroids, immunosuppressants, disease-modifying anti-rheumatic drugs (DMARDs) and newer biological agents such as tumor necrosis factor alpha (TNFα) and monoclonal antibodies. But, those drugs have severe side effects such as stomach irritation, malfunction of the kidney, urticaria, liver disorders, hematological abnormalities and gastrointestinal problems including ulcers, bleeding, heartburn, diarrhea, retention of fluid and perforation of stomach or intestine (6,7). Due to the chronic nature of arthritis and adverse reactions of NSAIDs and DMARDs, the advanced aged arthritic patients tend to rely on alternative treatments that are effective, less toxic and reduce the load of taking regular multiple medicines.

Moreover, synthetic drugs are very costly to develop. One drug requires approximately 3000-4000 compounds to be synthesized, screened and tested whose cost ranges from 0.5 to 5 million dollars. Hence, herbal drugs are favored over conventional medicines by patients as they have diminished the manifestations of illness and raised the worth of life (8).

Macropanax dispermus is a medicinal tree from the family of Araliaceae which is commonly found in evergreen forested areas. It is traditionally used for the treatment of indigestion, cough, menopausal fever, malarial fever, postpartum bathing, elimination of waste matter, and improvement of blood flow by ethnic people of Thailand, Myanmar (9, 10). In the previous researches, it was evident that its crude methanol extracts had a good amount of vitamin E, carotene, xanthophylls, tannins, phenolics and high amount of vitamin C (11).

Considering its medicinal properties, the leaves and stem barks of this plant were selected to conduct the investigations regarding the quantitative analysis of phytochemicals, anti-inflammatory and anti-arthritic properties of crude methanol extracts of *M. dispermus* leaves (MDML) and stem barks (MDMS) to establish an effective medicinal agent.

Materials and Methods

Chemicals

Methanol, gallic acid, quercetin, carrageenan, formalin, xylene, bovine serum albumin (BSA), and other chemicals used for the extraction, *in vivo*, and *in vitro* pharmacological tests were laboratory grade (Merck, Germany).

Collection and identification of the plant

The matured plant leaves and stem barks were collected in August 2018 from the Rangamati district (Chattogram hill tracts), Chattogram division of Bangladesh with the help of a famous local traditional healer. Then, it was distinguished as *M. dispermus* by a renowned taxonomist of Bangladesh, Dr. Shaikh Bokhtear Uddin, Professor, Department of Botany, University of Chittagong, Bangladesh. A specimen was deposited there under the herbarium no- sr20385.

Preparation of crude extracts

Plant materials (leaves and stem barks) were washed, chopped into small pieces, and semi-shed sun-dried for seven days. After drying, the plant materials were ground with a mechanical grinder facilitated by Pharmacological Research Laboratory, Department of Pharmacy, University of Chittagong, Bangladesh. Ground portions of the leaves (1.36 kg) and stem barks (493 g) of *M. dispermus* were soaked in 7.29 L and 2.60 L of methanol, respectively. After 13 days of occasional shaking, the solution was filtered and the filtrate was concentrated by evaporation method under reduced pressure by using a rotary evaporator (Stuart, UK) in the Pharmacological Research Laboratory, Department of Pharmacy, University of Chittagong, Bangladesh. The weight of the crude methanol extracts of *M. dispermus* leaves and stem barks was 28.50 g. and 7.66 g., respectively. The percentage (%) yield of the extract was calculated using the following equation (12):

% of yield of extract = (Weight of extract/ Weight of powder) × 100

The percentage of yield of crude methanol extracts of *M. dispermus* leaves and stem barks was 2.09% and 1.55%, respectively.

Experimental animals

Male and female Swiss albino mice weighing approximately 20-30 g were used for experimental purposes. All the animals were purchased from the animal house of Jahangirnagar University, Bangladesh. The mice were provided with nutritionally adequate diets (bought from the aforementioned animal house) and drinking water *ad libitum* throughout the study.

Study design

All the investigated extracts were used at the dose of 200 and 400 mg/kg of body weight of mice for the treatment group, whereas 1% tween-80 at 10 mL/kg was used for the negative control group. Indomethacin (10 mg/kg) served as a positive control in carrageenan- and formalin-induced paw edema tests. Prednisolone (10 mg/kg) served as a positive control in xylene-induced ear edema test.

Quantitative phytochemical screening

Determination of total phenol content

Total phenol content in MDML and MDMS was determined by using a previously described method with little modification (13). A volume of 1 mL of each of the extracts (1 mg/mL) was mixed with 5 mL of 10% Folin-Ciocalteu solution and 5 mL of Na_2CO_3 (7.5% w/v) solution. This mixture was vortexed for 15 sec and incubated at 25°C for 20 minutes for color development.

The absorbance of total phenol was taken at 760 nm using UV-Vis spectroscopy. To quantify total phenol content, the gallic acid standard curve was prepared and the results were displayed as milligrams of gallic acid equivalents (GAE)/g of dried extract. The concentration of total phenol content in the sample was determined by using the following equation:

$$A = \frac{c \times v}{m}$$

Here, A= Total phenol content (mg/g gallic acid equivalent), c= Concentration of gallic acid in mg/mL, v= Volume of the extract (mL), m= Mass of the extract (g)

Determination of total flavonoid content

Total flavonoids were determined using a previously described method with minor modification on the formation of a complex flavonoid-aluminum (14). One milliliter of 2% aluminium chloride-ethanol solution was mixed with 1 mL of each of the extracts (1 mg/mL). The mixture was incubated for 1 hour for yellow color development which indicated the presence of flavonoid. The absorbance was measured at 420 nm by using a UV-VIS spectrophotometer. To quantify total flavonoid content, quercetin standard curve was used and the results were expressed as quercetin equivalents (QE). The concentration of total flavonoid content in the sample was determined by using the following equation:

$$A = \frac{c \times v}{m}$$

Here, A= Total flavonoid content (mg/g quercetin equivalent), c= Concentration of quercetin in mg/mL, v= Volume of the extract (mL), m= Mass of the extract (g)

Determination of alkaloid content

Alkaloids were quantitatively determined according to the previously described method with minor modifications (15). Ten milliliters of 10% acetic acid in ethanol was added to 0.25 g of each of the extracts, covered and allowed to stand for 4 hours. The filtrate was then concentrated on a water bath. Concentrated ammonium hydroxide was added slowly to the extract until the precipitation was accomplished. The collected precipitates were washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed. The alkaloid content was determined using this formula:

%Alkaloid = (Final weight of extracts/Initial weight of extracts) × 100

In vivo anti-inflammatory activity

Carrageenan-induced paw edema test

This test was carried out using carrageenan as a phlogistic agent to induce paw edema in the right hind limb of mice (16). Six groups of mice (five mice to each group) were administered 1% tween-80 at 10 mL/kg, MDML and MDMS at the dose of 200 & 400 mg/kg orally and indomethacin at the dose of 10 mg/kg intraperitoneally. One hour after the extracts and thirty minutes after positive control administration, 100 µL of carrageenan (1% w/v in 0.9% normal saline) was injected subcutaneously into the right hind paw. Paw circumference was measured through a digital vernier caliper before the injection of carrageenan and thereafter at 1, 2, 3, and 4 hours. Paw edema was expressed as the change in paw circumference (mm) by using the following formula (1):

$$\text{Inhibition of edema (\%)} = \frac{Cc - Ct}{Cc} \times 100 \quad (1)$$

Here, Cc = Difference of paw circumference before and after carrageenan injection for control group at different time interval, Ct = Difference of paw circumference before and after carrageenan injection for test group at different time interval.

Formalin-induced paw edema test

The test was carried out using an established method with minor modifications (17)**.** Six groups of mice (five mice to each group) were administered 1% tween-80 at 10 mL/kg, MDML and MDMS at the dose of 200 & 400 mg/kg orally and indomethacin at the dose of 10 mg/kg intraperitoneally. One hour after the extracts and thirty minutes after positive control administration, the mice paw edema was induced by the subcutaneous injection of 50 µL of 2.5% formalin in 0.9% normal saline, into sub-planar tissue of the paws of mice. The paw circumference of mice was measured before and after formalin injection at 1, 2, 3, and 4 hours by digital vernier calipers. Paw edema was expressed as the change in paw circumference (mm) by using the formula (1).

Xylene-induced ear edema test

Xylene-induced ear edema model permitted the evaluation of anti-inflammatory steroids which was conducted by using the previously described method with slight modifications (18). Six groups of mice (five mice to each group) were administered 1% tween-80 at 10 mL/kg, MDML and MDMS at the doses of 200 & 400 mg/kg orally and prednisolone at the dose of 10 mg/kg intraperitoneally. Thirty minutes and fifteen minutes after the oral treatment of extracts and positive control administration to mice, respectively, the edema was induced by applying 30 µL of xylene to the inner surface of the right ear of mice using a syringe. Fifteen minutes of post-xylene application, the animals were anesthetized and both ears were cut off, sized, and weighed. The mean difference of the weight between the right and left ears was determined for each group and the percentage inhibition of edema was calculated as following (19):

$$\text{Inhibition of edema (\%)} = \frac{Wc - Wt}{Wc} \times 100$$

Here, Wc = Difference of ear weight in the negative control group, Wt = Difference of ear weight in the test group

In vitro anti-arthritic activity

Preparation of solutions

To prepare 0.5 mL of each test solution, 0.45 mL of 0.5% w/v aqueous solution of BSA was mixed with 0.05 mL of MDML and MDMS of different concentrations (1000, 500, 250, 125, and 62.5 µg/mL). The pH of the test solutions was adjusted to 6.3 using 1 N HCl.

To prepare 0.5 mL of standard solution, 0.45 mL of 0.5% w/v aqueous solution of BSA was mixed with 0.05 mL of diclofenac sodium of different concentrations (1000, 500, 250, 125, and 62.5 µg/mL). The pH of the standard solutions was adjusted to 6.3 using 1 N HCl.

To prepare 0.5 mL of test control solution, 0.45 mL of 0.5% w/v aqueous solution of BSA was mixed with 0.05 mL of distilled water. The pH of the test control solutions was adjusted to 6.3 using 1 N HCl.

To make 0.5 mL of product control solution, 0.05 mL of test solution was mixed with 0.45 mL of distilled water. The pH of the product control solutions was adjusted to 6.3 using 1 N HCl.

Distilled water was used as a blank to obtain the absorbance by using UV-Vis spectroscopy.

Experiment design

The evaluation of *in vitro* anti-arthritic activities of MDML and MDMS was conducted by using the "inhibition of protein denaturation" method (20-22). In this method, all the prepared solutions were incubated at 37°C for 20 minutes and then the temperature was increased to keep the solutions at 57°C for 3 minutes. Then the solutions were allowed to cool. After cooling, 2.5 mL of freshly prepared phosphate buffer (pH 6.3) was added to the previous solutions. The absorbance was measured by using a UV-Visible spectrophotometer at 416 nm. The percentage inhibition of BSA denaturation could be calculated as:

$$\text{\% Inhibition} = 100 - \left[\frac{A(t) - A(pc)}{A(tc)} \times 100\right]$$

Here, A(t)= Absorbance of test solution, A(pc)= Absorbance of product control, A(tc)= Absorbance of test control

Determination of median inhibitory concentration (IC_{50})

The relationship between concentration and inhibitory effect of MDML and MDMS was expressed as a median inhibitory concentration (IC_{50}). This represented the concentration of the sample required to inhibit 50% of the protein denaturation after a certain exposure time and determined by linear regression method from plotting percentage of inhibition against the correspondent logarithm of concentration. The concentration-inhibition data were transformed into a straight line through a trend line fit linear regression analysis (Microsoft Excel 2007); the IC_{50} value was derived from the best-fit line obtained.

Statistical analysis

All the data were expressed as mean ± SEM (standard error of mean). The results were analyzed statistically by one-way ANOVA followed by post hoc Dunnett's *t* test using statistical software Statistical Package for Social Science (SPSS, Version 16.0, IBM Corporation, NY). Results $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ were considered statistically significant as compared to control.

Results

Quantitative phytochemical screening

In the present study, it was found that MDML and MDMS contained a considerable quantity of phenol, flavonoid, and alkaloid. The current investigation showed that total phenol content of MDML and MDMS was 15.67 ± 1.5 and 23.56 ± 1.06 mg of gallic acid equivalent per g of each of the extracts, respectively. Total flavonoid content of MDML and MDMS was 11.8 ± 0.42 and 10.07 ± 0.85 mg of Quercetin equivalent per g of each of the extracts, respectively. Gallic acid and quercetin equivalent concentration was obtained using the expression from the calibration curve of gallic acid and quercetin (Figure 1). The alkaloid content of MDML and MDMS was determined to be 64.71 ± 0.94% and 59.24 ± 0.6%, respectively (Table1).

In vivo anti-inflammatory activity

Carrageenan-induced paw edema test

In the current study of anti-inflammatory activity analysis of the investigated extracts by using carrageenan-induced paw edema test, negative control group showed the increase in paw edema which extended up to 3 hours, and then the paw edema was decreased slightly. Indomethacin, positive control inhibited paw edema slightly and started inhibiting paw edema from 3rd hour by 66.67% to 4th hour by 73.53% significantly ($P<0.001$). MDMS showed an excellent effect in this method which inhibited paw edema from 26.19% to 58.52% after its administration till the 4th hour at 200 mg/kg. MDMS increased the paw edema inhibition at higher dose (400 mg/kg) which continued up to 4th hour of its administration from 28.57% to 76.47% significantly ($P<0.001$) (Table 2).

Formalin-induced paw edema test

In formalin-induced paw edema test, negative control group showed the increase in edema which slightly decreased from the 3rd hour of the post-injection period, whereas positive control group showed the inhibition of paw edema from the 1st hour to the 4th hour of the post-

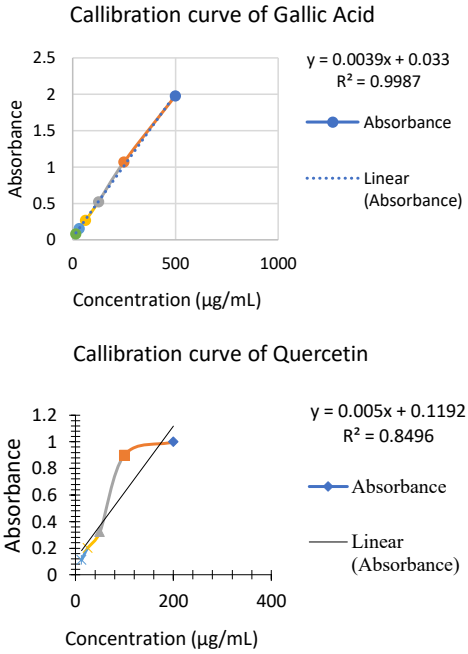

Figure 1. Calibration curve of gallic acid and quercetin.

injection period from 20.34% to 86.79%, significantly ($P<0.001$). In this study, MDMS also showed good effect which demonstrated paw edema inhibition effect from 2nd hour significantly ($P<0.001$) which was ranged from 24.59% to 50.94% (200 mg/kg) and 42.62% to 80.75% (400 mg/kg), respectively (Table 3).

Xylene-induced ear edema test

In xylene-induced ear edema test, negative control group showed much increase in-ear edema whereas the positive control, prednisolone reduced the edema by 81.66% during 15 minutes of post-injection significantly ($P<0.001$). MDML also showed an excellent potential to reduce the edema by 74.62% at the low dose and 82.49% at the high dose significantly ($P<0.001$) (Table 4).

In vitro anti-arthritic activity

In the current study of *in vitro* anti-arthritic activity, positive control showed much higher inhibition of protein denaturation from 96.61% to 62.71% from the highest concentration to the lowest concentration, whereas the negative control did not show any inhibition of protein denaturation. MDMS was observed to inhibit protein denaturation effectively which was ranged from 61.02% to 0% (Table 5).

Discussion

From the ancient period, herbal medicines have been used as the major therapy in medical practices. The use of traditional medicine persists today because of its medicinal value as well as a part of cultural beliefs in many countries of the world and the toxicity and side effects of conventional medicines (23). In this regard, medicinal plant *M. dispermus* was investigated to discover its potential as an effective anti-inflammatory and anti-arthritic agent.

Table 1. Total phenol, flavonoid, and alkaloid content of crude methanol extracts of *M. dispermus* leaves and stem barks

Group	Total phenol mg/g of extracts (GAE)	Total flavonoid mg/g of extracts (QE)	Total Alkaloid content (%)
MDML (1 mg/mL)	15.67 ± 1.50	11.80 ± 0.42	64.71 ± 0.94
MDMS (1 mg/mL)	23.56 ± 1.06	10.07 ± 0.85	59.24 ± 0.60

MDML, Crude methanol extracts of *M. dispermus* leaves; MDMS, Crude methanol extracts of *M. dispermus* stem barks; GAE, Gallic acid equivalent; QE, Quercetin equivalent. Results were expressed as mean ± SEM.

Table 2. Anti-inflammatory activity of crude methanol extracts of *M. dispermus* leaves and stem barks by using carrageenan-induced paw edema test

Group	Dose (mg/kg)	Pre-injection paw circumference (mm)	Post-injection paw circumference in mm (% of edema inhibition)			
			1 h	2 h	3 h	4 h
Control	10 mL/kg	9.80 ± 0.58	14 ± 0.45	14.40 ± 0.51	14 ± 0.45	13.20 ± 0.37
Indomethacin	10	10.20 ± 0.37	13 ± 0.32 (33.33%)	13.60 ± 0.43 (26.09%)	11.60 ± 0.24** (66.67%)	11.10 ± 0.33** (73.53%)
MDML	200	9.60 ± 0.24	12.80 ± 0.37 (23.81%)	14.20 ± 0.58 (6.52%)	12.80 ± 0.58 (23.81%)	11.80 ± 0.37* (35.29%)
	400	10.20 ± 0.37	13.20 ± 0.58 (28.57%)	12.90 ± 0.46 (41.30%)	12.10 ± 0.60* (54.76%)	11.30 ± 0.44** (67.65%)
MDMS	200	10.30 ± 0.3	13.40 ± 0.4 (26.19%)	13.70 ± 0.54 (26.09%)	12.60 ± 0.51 (45.24%)	11.70 ± 0.34* (58.52%)
	400	10.60 ± 0.24	13.60 ± 0.24 (28.57%)	13.10 ± 0.33 (45.65%)	11.90 ± 0.24* (69.05%)	11.40 ± 0.19** (76.47%)

MDML, Crude methanol extracts of *M. dispermus* leaves; MDMS, Crude methanol extracts of *M. dispermus* stem barks.
Results were expressed as mean ± SEM.
Results were significant at *$P<0.05$, **$P<0.01$ and ***$P<0.001$ as compared to negative control group.

Table 3. Anti-inflammatory activity of crude methanol extracts of *M. dispermus* leaves and stem barks by using formalin-induced paw edema test

Group	Dose (mg/kg)	Pre-injection paw circumference (mm)	Post-injection mean paw circumference (mm) (% of edema inhibition)			
			1 h	2 h	3 h	4 h
Control	10 mL/kg	10.60 ± 0.24	16.50 ± 0.22	16.70 ± 0.2	16.10 ± 0.24	15.90 ± 0.19
Indomethacin	10	10.30 ± 0.3	15 ± 0.32* **(20.34%)**	13.10 ± 0.24*** **(54.1%)**	11.60 ± 0.19*** **(76.36%)**	11 ± 0.27*** **(86.79%)**
MDML	200	10.20 ± 0.34	16 ± 0.45 **(1.69%)**	14.90 ± 0.4** **(22.95%)**	13.90 ± 0.48*** **(32.73%)**	13.30 ± 0.49*** **(41.51%)**
	400	10.40 ± 0.24	15.30 ± 0.44 **(16.95%)**	13.60 ± 0.37*** **(47.54%)**	12.70 ± 0.20*** **(58.18%)**	11.60 ± 0.24*** **(77.37%)**
MDMS	200	10.80 ± 0.37	16.40 ± 0.29 **(5.08%)**	15.40 ± 0.19* **(24.59%)**	14.30 ± 0.30** **(36.36%)**	13.40 ± 0.37*** **(50.94%)**
	400	10.40 ± 0.24	15.70 ± 0.41 **(10.17%)**	13.90 ± 0.33*** **(42.62%)**	12.20 ± 0.34*** **(67.27%)**	11.42 ± 0.28*** **(80.75%)**

MDML, Crude methanol extracts of *M. dispermus* leaves; MDMS, Crude methanol extracts of *M. dispermus* stem barks.
Results were expressed as mean ± SEM.
Results were significant at **P* < 0.05, ***P* < 0.01 and ****P* < 0.001 as compared to negative control group.

Table 4. Anti-inflammatory activity of crude methanol extracts of *M. dispermus* leaves and stem barks by using xylene-induced ear edema test

Group	Dose (mg/kg)	Mean difference in ear weight	Inhibition of edema (%)
Control	10 mL/kg	45.80 ± 3.08	-
Prednisolone	10	8.40 ± 1.50***	81.66
MDML	200	11.62 ± 0.50***	74.62
	400	8.02 ± 0.58***	82.49
MDMS	200	15.32 ± 3.23***	66.55
	400	8.83 ± 1.42***	80.73

MDML, Crude methanol extracts of *M. dispermus* leaves; MDMS, Crude methanol extracts of *M. dispermus* stem barks; GAE, Gallic acid equivalent; QE, Quercetin equivalent.
Results were expressed as mean ± SEM.
Results were significant at **P* < 0.05, ***P* < 0.01 and ****P* < 0.001 as compared to negative control group.

Table 5. Anti-arthritic activity of crude methanol extracts of *M. dispermus* leaves (MDML) and stem barks (MDMS)

Group	Concentration (µg/mL)	% of Inhibition of Protein Denaturation	IC_{50} (µg/mL)
Control	-	0	-
Diclofenac Sodium	1000	96.61	19.26
	500	91.53	
	250	81.36	
	125	74.58	
	62.5	62.71	
MDML	1000	45.76	1317.51
	500	33.90	
	250	28.81	
	125	20.34	
	62.5	3.39	
MDMS	1000	61.02	631.59
	500	45.76	
	250	30.51	
	125	1.69	
	62.5	0	

MDML, Crude methanol extracts of *M. dispermus* leaves; MDMS, Crude methanol extracts of *M. dispermus* stem barks.

In carrageenan-induced paw edema model, negative control did not show any inhibition of paw edema, whereas indomethacin inhibited the paw edema significantly from the 3rd hour. When compared to negative control group, MDML showed inhibition of paw edema from the 3rd hour at low dose and from the 1st hour at high dose. Its low dose possessed mild effect whereas its high dose showed significant and high potential as an anti-inflammatory agent. MDMS showed significant inhibition of paw edema from the 1st hour which continued up to 4th hour as compared to negative control group. It showed a moderate anti-inflammatory effect at a low dose, and its higher dose

showed an excellent anti-inflammatory as well as an anti-edematogenic effect. The anti-inflammatory mechanism of MDML and MDMS might be related to the inhibition of prostaglandins and nitric oxide synthesis, as described for the anti-inflammatory mechanism of indomethacin in inhibiting the inflammatory process, induced by carrageenan (24).

In formalin-induced paw edema test, negative control group showed no inhibition, whereas indomethacin showed the inhibition slightly at the first two hours of post-injection which was predominant during the last two hours of post-injection period significantly. When compared to negative control group, MDML showed significant inhibition of formalin-induced paw edema at both doses from the 2nd hour of post-injection. MDML showed a moderate anti-inflammatory effect at the low dose (200 mg/kg) which showed high potential to inhibit the inflammatory edema at the high dose (400 mg/kg). When compared to negative control group, MDMS also displayed significant inhibition of formalin-induced paw edema from the 2nd hour of post-injection of formalin. MDMS at the low dose showed moderate and at the high dose, it exhibited excellent anti-inflammatory activity up to 4th hour of the post-injection period. This indicated that it had a strong inhibitory effect on the proliferation of fibroblasts and also probably connective tissue modulation effect. But, it did not reflect a good neurogenic effect in the reduction of formalin-induced inflammation.

In xylene-induced ear edema test, negative control group asserted much increase in the ear edema whereas prednisolone showed the highest inhibition of ear edema, since it acted as a steroid agent to inhibit the production of phospholipase A_2 (25). It was quite evident that it would reduce the xylene-induced neurogenic inflammation successfully. When compared to negative control group, MDML and MDMS showed an excellent potential to decrease the xylene-induced ear edema at both doses significantly ($P < 0.001$). MDML at the high dose (400 mg/kg) showed more effectiveness in decreasing ear edema than that of positive control group and proved that it might be an excellent alternative of prednisolone. MDMS also could be used as a highly active anti-inflammatory agent at the higher dose.

In the current study of anti-arthritic activity, diclofenac sodium was used as a reference standard which is used conventionally as an anti-arthritic agent. It showed the highest inhibition of thermally induced denaturation of BSA at the highest concentration of 1000 µg/mL. It showed a decrease in the inhibition of protein denaturation with the decreasing concentration. MDML showed a very mild and concentration-dependent effect as an antiarthritic agent with comparison to control and standard. MDMS also showed less inhibition of BSA denaturation than that of standard solution at the lowest concentration. When compared to control and standard, MDMS showed moderate effectiveness as an anti-arthritic agent at a higher concentration. Considering the IC_{50} value, MDMS was evident as more as a potent herbal remedy for arthritis. The findings of the present study did not associate albumin denaturation in the inflammatory process, but there might be a relevant connection to studies made on the antigenicity of albumin aggregates (26).

Previous studies reported that some plants that were rich in alkaloids and flavonoids, showed anti-inflammatory as well as anti-arthritic activity (27-29). In the current research, quantitative analysis showed a considerable and different amount of phenols, flavonoids, and a higher percentage of alkaloids in MDML and MDMS. The presence of those phytoconstituents might contribute to their different pharmacological activities.

Conclusion

MDML and MDMS contains a considerable amount of phenols, flavonoids, and alkaloids and are excellent anti-inflammatory and anti-arthritic agents. This research work can help formulate a potent herbal entity that may be used in the treatment of inflammation and arthritis. Further research must be conducted to isolate and identify its active compounds which will surely be responsible for their pharmacological activities.

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Author's contributions

MKH designed the research experiment and conception. SRA collected and processed the plant material. SRA and MKS carried out the laboratory work. Data analysis and interpretation were aided by the MRI and SA. NMP and MRI made the necessary corrections in the write up and critically revised the manuscript, gave final approval for the submission of revised version. Finally, all authors read the final version and gave their consent to submit.

Conflict of interests

All authors declare that they have no competing interest.

Ethical considerations

All authors hereby declare that "Principle of laboratory

animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All proposed research protocols have been examined and approved by the ethical committee of University of Chittagong, Bangladesh under the approval no- cc98056.

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