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Phytochemical and pharmacological investigations of different extracts of leaves and stem barks of *Macropanax dispermus* (Araliaceae): a promising ethnomedicinal plant

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Abstract

Background: *Macropanax dispermus* is traditionally used to treat various diseases by ethnic people. The present research reports the pharmacological properties with phytochemical profiling of the crude extracts of *M. dispermus* leaves (MDML), its n-hexane (MDHL), carbon tetrachloride (MDTL), chloroform (MDCL), ethyl acetate (MDEL), and aqueous (MDAL) fractions, and crude methanol extracts of its stem barks (MDMS). The in vitro thrombolytic activity was done on human erythrocytes whereas the cytotoxic activity was done by brine shrimp lethality assay. The in vivo analgesic activity was examined by acetic acid-induced writhing, tail immersion, and formalin-induced paw licking method. In contrast, antipyretic activity was done by the brewer's yeast-induced pyrexia method.

Results: MDHL and MDMS showed 37.05% and 42.21% of significant ($p < 0.01$) thrombolytic activity, respectively. MDCL and MDMS showed the lower LC_{50} values of 23.15 and 37.11 µg/ml during cytotoxicity test, respectively. In acetic acid writhing method, MDTL and MDEL showed significant ($p < 0.001$) inhibition of writhing by 79.34% and 80.17%, respectively. MDMS showed significant ($p < 0.001$) maximal possible effect (%MPE) of 45.95%, 62.26%, 65.79%, 89.69% and elongation of time in pain reaction of 48.53%, 60.28%, 58.76%, and 70.14% at 30, 60, 90, and 120 min intervals, respectively. MDML at 400 mg/kg exhibited significant ($p < 0.001$) 82.72% of inhibition of pain at the late phases. MDEL at 400 mg/kg of dose exhibited significant ($p < 0.001$) reduction of rectal temperature by 36.31%, 62.42%, 89.81%,, and 96.82% at 1, 2, 3, and 4 h intervals, respectively.

Conclusion: The current research suggests that the plant extracts possess potential thrombolytic, cytotoxic, analgesic, and antipyretic activities.

Keywords: *Macropanax dispermus*, Phytochemicals, Thrombolytic, Cytotoxic, Analgesic, Antipyretic

Background

From the ancient period, natural remedies have been popular with people due to their excellent medicinal properties which can treat complicated diseases without showing any side effects at minimum expense. The medicinal plants contain different types of naturally occurred chemical compounds that contribute to their medicinal properties. With the advancement of scientific research, the researchers have been successful to detect

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various phytochemical compounds in medicinal plants which are proved to exhibit different types of physiological activities and are used for prophylactic purposes [1].

Thrombosis is a lethal disease that causes vascular blockage and while recovering it causes fatal consequences, such as cerebral or myocardial infarction and even death [2]. Tissue plasminogen activator (t-PA), alteplase, anistreplase, urokinase, and streptokinase (SK), and recombinant t-PA therapies are currently used for the treatment of thrombosis [3]. The post-therapeutic adverse effects of these medicines are extremely significant which may cause further deterioration of a patient's health conditions [4]. These include intracranial hemorrhage [5], spontaneous pulmonary hemorrhage [6], angioedema [7], slow reperfusion rate, and frequent early re-occlusions which have decreased the use of thrombolytic drugs in many cases [8]. Cancer is another fatal disease that can cause abnormal cell growth, uncontrolled cell proliferation, differentiation, and ultimate death of the cells. The modern conventional treatment suggests chemotherapy, radiotherapy, or surgery fight against it which exhibited several adverse effects [9]. Natural cytotoxic agents may damage the cancerous cell and contribute to the treatment of cancer. Pain or algesia is generally an unpleasant sensation associated with tissue damage which is initially protective but responsible for many discomforts leading to adverse effects [10]. Analgesics are drugs used to treat or reduce the pain that act on the peripheral or central nervous system to selectively relieve pain without significantly altering consciousness [11]. The classical analgesic drugs are notably opiates and non-steroidal anti-inflammatory drugs (NSAIDs) [12]. Fever or pyrexia is an elevated body temperature above the normal level characterized by an increase in the thermoregulatory set-point, which results from the interaction of the central nervous and immune system. Fever is the body's natural defense mechanism against infectious agents that can damage the tissue [13]. NSAIDs also possess antipyretic activity. But NSAIDs exhibited severe adverse effects like ulceration, gastrointestinal bleeding, respiratory distress, drowsiness, nausea and along with these side effects opiates also exhibited constipation, physical dependence, addictive potential etc. [14].

Herbal medicines as the major remedy in the traditional system of medicine have been used in medical practices since antiquity [15]. In ancient literature, about 500 medicinal plants are mentioned and about 800 plants are used in the traditional system of medicine [16]. With the introduction of conventional medicines, scientists started to doubt the safety of traditional medicine because of a lack of proper scientific data. In the nineteenth century, human began to isolate the active principles of medicinal plants and was successful to invent quinine from Cinchona bark [17]. Such discoveries convinced scientists to rely on this alternative form of medicine and proceed to further investigations. Moreover, it has started to become popular with common people because of more effectiveness, fewer health hazard risks, and cheaper rates than conventional medicine. Thus the impact of phytochemistry led to the discovery of many of the effective conventional medicinal agents which were derived from plant sources and introduced anticancer drugs like vincristine, vinblastine, and paclitaxel [18], narcotic analgesics like morphine [19], and anti-malarial drugs like quinine and artemisinin [20]. Therefore, the current study focuses on the phytochemical and pharmacological investigations of an ethnomedicinal plant *Macropanax dispermus*.

Macropanax dispermus is a tree from the family of *Araliaceae*, and it is grown in evergreen forested areas. It was traditionally used for the treatment of digestion, postpartum bathing, eliminate waste matter, improve blood flow, cough, menopausal fever, and malarial fever by ethnic people of Thailand, Myanmar [21, 22]. Previous studies reported that its crude methanol extracts showed a good amount of vitamin E, carotene, xanthophylls, tannins, phenolics, and the highest amount of vitamin C by using the β-carotene bleaching method [23].

So, the current research was conducted to investigate the phytochemical contents, thrombolytic, cytotoxic, analgesic, and antipyretic activity of the crude methanol extracts of the leaves and stem barks of *M. dispermus* and the solvent fractions of the crude methanol extracts of its leaves.

Methods

Chemicals

Methanol, n-hexane, carbon tetrachloride, chloroform, ethyl acetate, and other chemicals used for the extraction, solvent–solvent partitioning of plant materials, in vitro, and in vivo 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 with the help of a famous local traditional healer. Then it was identified by a renowned taxonomist 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 powdered with a mechanical grinder. Powder 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 at the temperature below 50 °C by using a rotary evaporator (Stuart, UK). The weight of the crude methanol extracts of *M. dispermus* leaves and stem barks was 28.50 gm. and 7.66 gm., respectively. The percentage (%) yield of the extract was calculated using the following equation [14]:

$$\% \text{ Yield of extract} = \frac{\text{Weight of extrcated material}}{\text{Weight of crude powder}}$$

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

Solvent–solvent partitioning

Crude methanol extracts of *M. dispermus* leaves were undergone solvent–solvent partitioning according to the protocol designed by Kupchan and Tsou and modified version of Wagenen et al. by using the solvents of n-hexane, carbon tetrachloride, chloroform, and ethyl acetate consecutively [24, 25].

Qualitative phytochemical screening

The preliminary phytochemical screening was done for evaluating the qualitative detection of terpenoids, flavonoids, saponins, phenol and tannins, phlobatannins, steroids, anthraquinones, alkaloids, glycosides, cardiac glycosides, resins, carbohydrates, proteins, fat and oil, and coumarin by using standard procedure [26–28]. The color intensity or the precipitate formation was used as analytical responses to these qualitative tests.

Experimental animals

Male Swiss Albino mice weighing approximately 20–30 g were used for experimental purposes. They were placed in standard propylene cages and familiarized under the controlled conditions (room temperature of 25 ± 2 °C, relative humidity of 60%-70% for 14 days) and operated with a 12 h light/dark cycle with food pellets. The mice were provided with a nutritionally adequate diet and drinking water ad libitum throughout the study. Diethyl ether anesthesia was employed on mice to euthanize. All the mice were sacrificed at the end of each experiment by using diethyl ether anesthesia. All segments of this report adhere to the ARRIVE Guidelines for reporting animal research. All experiments have been examined and approved by the ethical committee under approval no-cc98056.

Acute toxicity study

An acute toxicity study was conducted following the previously described method [29]. Each group comprises five Swiss albino mice and they fasted overnight before extract administration. Each group of animals was administered oral doses of 1000, 2000, 3000, and 4000 mg/kg of body weight of each of the extracts. After the administration of plant extract, they were restrained from food for further 3–4 h. Each animal was observed for the first 30 min, then for the first 24 h, and thereafter for 3 days. The mice were observed for any changes in the skin, fur, eyes, mucous membrane, respiration rate, circulatory rate, the central and autonomic nervous system at least once a day. The effective dose would be one-tenth of the median lethal dose (LD_{50}).

Experimental design for in vivo testing

During the evaluation of analgesic activity, 16 groups of mice were used for each investigation and five mice were selected for each group. Group (I) was treated as control (1% tween-80 10 ml/kg), Group (II) was for standard (Diclofenac sodium 50 mg/kg used in acetic acid writhing study, and morphine sulfate 10 mg/kg served as the standard in both tail immersion and formalin-induced paw licking method) and others group were used for the administration of crude methanol extract of *M. dispermus* stem bark and leaves and its different extracted fractions at the dose of 200 and 400 mg/kg. For brewer's yeast-induced antipyretic study, sixteen groups of mice were selected, numbered, five mice were assigned to each group. Group (I) was treated as control (1% tween-80 10 ml/kg), Group (II) was for standard Paracetamol (150 mg/kg) and other groups were used for the administration of crude methanol extract of *M. dispermus* stem bark and leaves and its different extracted fractions at the dose of 200 and 400 mg/kg. After each experimental period, all the treated mice were sacrificed using diethyl ether anesthesia.

In vitro studies of *Macropanax dispermus* extracts

Evaluation of thrombolytic activity

Blood specimen Venous blood samples were drawn from 30 male and female healthy volunteers (age 18–26 years) who have no recent history of oral contraceptive and anticoagulant therapy. Ten blood samples were used for each concentration of each plant extracts. After that, 6 ml of venous blood were drawn from each volunteer. Blood was collected and preserved by an expert senior medical technologist. A consent form was filed up for each volunteer for future reference.

Study design Experiments for clot lysis were carried out as reported earlier [30]. About 500 µl of blood was taken into each pre-weighed Eppendorf tube and allowed to incubate at 37 °C for 45 min. After clot formation, fluid was completely released from each Eppendorf tubes and

the clot weight was determined by subtracting the weight of the clot containing tube from the weight of the tube alone following the equation below:

$$\text{Weight of Clot} = \text{Weight of Clot Filled Eppendorf} - \text{Weight of Empty Eppendorf}$$

As standard, 100 µl of Streptokinase (SK) and, as a negative non-thrombolytic control, 100 µl of distilled water (DW) along with 100 µl of each sample of different concentrations (10 mg/ml, 8 mg/ml, 6 mg/ml, 3 mg/ml, 1.5 mg/ml) were separately added to the Eppendorf tubes. Then the Eppendorf tubes were incubated at 37 °C for 90 min. After incubation, the released fluid was discarded and tubes were again weighed to observe the difference in weight after clot disruption. Finally, the percentage (%) of clot lysis was determined as follows:

$$\%\ \text{Clot Lysis} = \frac{\text{Weight of Clot Before Lysis} - \text{Weight of Clot Lysis}}{\text{Weight of Clot Before Lysis}} \times 100$$

Evaluation of cytotoxic activity

Preparation of seawater Exactly 38 gm sea salt (without iodine) was dissolved in 1 L of distilled water and filtered off to get a clear solution. The pH of the seawater was maintained between 8.0–8.5 by applying 1 N NaOH solution [31].

Hatching of brine shrimp *Artemia salina* leach (brine shrimp eggs) was collected from pet shops in Chittagong, used as the test organism. Seawater was taken in the small tank and shrimp eggs were added to the seawater in the tank. After 2 days (48 h) of hatching, the eggs would be matured as nauplii. The oxygen was supplied constantly through the oxygen pump during hatching time. The hatched shrimps were attracted to the light (phototaxis) and so nauplii free from eggshell was collected from the illuminated part of the tank. The nauplii were taken from the fish tank by a pipette.

Study design Five milliliters of each of the plant extract solutions of different concentrations (1000, 500, 250, 125, 62.5 µg/ml) was added to 5 ml of seawater containing 10 nauplii. After 24 h, the Petri dishes were inspected using a magnifying glass against a black background and the number of survived nauplii in each was counted. The mortality endpoint of this bioassay was determined as the absence of controlled forward motion during 30 s of observation. From these data, the percentage (%) of the mortality of the brine shrimp nauplii was calculated for each concentration from the following equation [32]:

$$\%\ \text{Mortality} = \frac{N\text{d}}{N} \times 100$$

Here N_d = Number of dead nauplii, N = Number of nauplii taken.

Determination of median lethal concentration (LC_{50})

The LC_{50} value represented the concentration of the extract that produced death in half of the brine shrimp nauplii after a certain exposure time and was determined by the linear regression method from plotting % of mortality against the correspondent concentration of the extracts. An approximate linear correlation was observed when concentration versus the percentage of mortality was plotted on the graph paper and the concentration–response data were transformed into a straight line utilizing a trend line fit linear regression analysis (Microsoft Excel 2007). The LC_{50} values were derived from the best-fit line obtained.

In vivo studies of *Macropanax dispermus* extracts

Evaluation of analgesic activity

Acetic acid-induced writhing method This method was an analgesic behavioral observation assessment method that demonstrated a noxious stimulation in mice. This study was carried out using the method of Koster as modified by Dambisya and Lee [33, 34]. Fifteen minutes after administration of standard and 30 min after administration of the extract, 0.7% glacial acetic acid (10 ml/kg) was injected intraperitoneally (IP) to all the mice to induce pain characterized by abdominal constrictions or writhes. 5 min later, each mouse of all groups was observed to count the number of writhes for 20 min carefully and recorded. After each observation period, all the treated mice were euthanized using diethyl ether anesthesia. The percentage inhibition against abdominal writhing was used to assess the degree of analgesia and was calculated using the formula:

$$\%\ \text{of Pain Inhibition} = \frac{N_c - N_t}{N_c}$$

Here N_c = number of writhings in the control group, and N_t = number of writhings in the treatment group.

Tail immersion method This was a thermal method that was performed to evaluate the central analgesic property of the investigated extracts. The method described by Di Stasi et al. [35] was used for this experiment. Before 30 min of treatment about 2–3 cm of the tail of each of the mice was dipped into a water bath containing warm water maintained at a temperature of 50 ± 1 °C and the time taken for the mice to withdraw its tail from the warm water was recorded. The animals, which showed a flicking response within 3–5 s, were selected for the study. A cut-off period of 15 s was determined to avoid damage to the tail. After baseline, the treated mice were tested at 30, 60, 90, and 120 min after drug administration [36]. While measurements were being made, animals were briefly immobilized by wrapping them gently. After each observation period, all the treated mice were euthanized using diethyl ether anesthesia. The percentage of the Maximal Possible Effect (% MPE) was calculated using the following equation [37]:

$$\%\ \text{of MPE} = \frac{\text{Post-drug Latency} - \text{Pre-drug Latency}}{\text{Cut off time} - \text{Pre-drug Latency}} \times 100$$

The percentage of time elongation was calculated from the following equation [38]:

$$\%\ \text{of Elongation of Latency} = \frac{\text{Latency of Treatment} - \text{Latency of Control}}{\text{Latency of Treatment}} \times 100$$

Formalin-induced paw licking method This was a persistent-pain model that was used to assess both central and peripheral analgesic effects of the investigated extracts. This analgesic assay was done using a previously described method [39]. After sixty minutes of administration of control, standard, and investigated extracts, 20 µl of 1% formalin solution was injected through the subplantar route into the right hind paw of each mouse. The time (in seconds) spent licking or biting the injected paw indicated pain and was recorded. After subplantar injection of formalin, the responses of the mice were noticed for the first 5 min (early or neurogenic phase) and 15–30 min (late or inflammatory phase). After each observation period, all the treated mice were euthanized using diethyl ether anesthesia. The percentage (%) of pain inhibition was calculated using the following formula:

$$\%\ \text{Inhibition of Pain} = \frac{\text{Reaction Time (Control)} - \text{Reaction Time (Treatment)}}{\text{Reaction Time (Control)}} \times 100$$

Evaluation of antipyretic activity

The antipyretic effect was assessed by using the brewer's yeast-induced pyrexia method described by Adams et al. [40]. Before experimentation, the rectal temperature of mice was recorded by a digital thermometer. Hyperthermia was induced in mice by subcutaneous injection of 15% aqueous suspension of brewer's yeast (10 ml/kg) in the back below the nape of the mice and the injected site was massaged to spread. Pre-drug temperatures were taken 24 h after the yeast injection to determine the pyretic response of yeast. Animals with 1 °F or more elevation in body temperature were used. The temperatures were recorded at 1, 2, 3, and 4 h intervals after the drug treatment. After each observation period, all the treated mice were euthanized using diethyl ether anesthesia. The percentage (%) reduction of rectal temperature could be calculated by the following formula [41]:

$$\%\ \text{Reduction of rectal temperature} = \frac{B - C}{B - A} \times 100$$

Here A = Normal body temperature, B = Rectal temperature at 24 h after yeast administration, and C = Rectal temperature after drug administration at a different time interval.

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 test using statistical software "Statistical Package for Social Science" (SPSS, Version 16.0, IBM Corporation, NY). Results below $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ were considered statistically significant compared to control.

Results

Qualitative phytochemical screening

The current study was carried out to screen the presence or absence of preliminary phytochemicals in the investigated extracts which showed the presence of different types of phytochemicals in the extracts which are demonstrated in Table 1.

Acute toxicity study

No mice showed any abnormal changes such as reduced motor activity, restlessness, convulsions, coma, diarrhea, and lacrimation at the experimental doses of each of the investigational extracts. No mice were dead at the

Table 1 Preliminary screening of phytochemicals of the investigated extracts of *M. dispermus* leaves and stem barks

Phytochemicals	Methods	MDML	MDHL	MDTL	MDCL	MDEL	MDAL	MDMS
Terpenoids	Salkowski's test	+	−	−	+	−	+	+
	Liebermann–Burchard's test	++	−	−	++	−	++	++
Flavonoids	Alkaline reagent test	−	−	−	−	−	+	+
	Zinc–hydrochloric acid reduction test	++ (Flavonol)	+ (Flavone)	−	+ (Flavono−ids)	−	−	++ (Flavono−l)
	Lead acetate solution test	+	−	−	−	−	+	++
	Sulfuric acid test	++	−	−	−	−	+	++
Saponins	Foam test	+++	−	−	−	−	+++	++
Phenols and tannins	Ferric chloride test	+	−	−	−	−	−	+(Phenol)
Phlobatannins	Hydrochloric acid test	+	−	−	−	−	−	++
Steroid	Salkowski's test	+	−	−	−	−	−	−
	Liebermann–Burchard's test	−	−	−	−	−	−	−
Anthraquinones	Acid test	++	+	−	−	−	−	++
	Hydroxy-anthraquinone test	++	−	−	−	−	−	+
Alkaloids	Mayer's test	++	−	−	−	−	−	+
	Wagner's test	++	−	−	−	−	−	+
	Hager's test	++	−	−	−	−	−	+
Glycosides	Sodium hydroxide reagent test	−	−	−	+	−	+	+
	Liebermann's test	+	−	−	+	−	−	−
	Salkowski's test	+	−	−	−	−	−	+
Cardiac glycosides	Keller–Killiani test	+	−	−	−	−	+	++
	Baljet's test	+	−	−	+	−	+	++
Resins	Acetone test	−	−	−	−	−	−	+
Carbohydrates	Benedict's test	−	−	−	−	−	−	++
	Molisc-h's test	+	−	+	−	−	−	++
	Iodine test	−	−	−	−	−	−	−
	Fehling's test	+	−	−	−	−	−	++
Proteins	Biuret test	+	−	−	+	+	−	−
	Nitric acid test	++	−	−	−	−	+	+
Fats and fixed oils	Copper sulfate test	+	−	+	−	−	−	+
Coumarin	Sodium hydroxide test	−	−	−	−	−	−	−

"+"=Present, "++"=More presence, "+++"=Very much presence, "−"=Absent, MDML=Crude methanol extract of *M. dispermus* leaves, MDHL=n-hexane fraction of crude methanol extracts of *M. dispermus* leaves, MDTL=Carbon tetrachloride fraction of crude methanol extracts of *M. dispermus* leaves, MDCL=Chloroform fraction of crude methanol extracts of *M. dispermus* leaves, MDEL=Ethyl acetate fraction of crude methanol extracts of *M. dispermus* leaves, MDAL=Aqueous fraction of crude methanol extracts of *M. dispermus* leaves, MDMS=Crude methanol extract of *M. dispermus* stem bark

experimental doses. So, the LD_{50} was investigated to be greater than 4000 mg/kg.

In vitro studies of *Macropanax dispermus* extracts

Evaluation of thrombolytic activity

The thrombolytic activity of the investigated extracts was determined as a part of the exploration of cardio-protective drugs from plant reserves. The results conferred in Table 2 showed that among multiple extracts, MDMS, MDML, MDHL, and MDCL produced significant ($p<0.001$) clot lysis (%) in a concentration-dependent manner. Among them, MDMS and its MDHL fraction showed 42.21% and 37.05% clot lysis at the dose of 10 mg/ml respectively, compared to others.

Evaluation of cytotoxic activity

The level of toxicity against brine shrimp was classified as toxic having an LC_{50} value less than 1000 µg/ml and non-cytotoxic having an LC_{50} value greater than 1000 µg/ml [42]. The brief results displayed in Table 3 indicate that the MDCL, MDEL, MDAL, and MDMS produced high cytotoxicity with the lowest LC_{50} values whereas MDML and its MDHL, MDTL fractions were observed to be non-cytotoxic. Among these extracts, MDCL fraction and crude MDMS showed cytotoxicity with the lowest LC_{50} values of 23.15 and 37.11 µg/ml, respectively, compared to others. However, positive control vincristine sulfate was observed to be a highly cytotoxic substance.

Table 2 Thrombolytic activity of the investigated extracts of *M. dispermus* leaves and stem barks

Group	Mean ± SEM % of clot lysis
Control	3.71 ± 0.44
Standard (streptokinase)	64.72 ± 1.88***
MDML (10 mg/ml)	27.36 ± 3.83**
MDML (8 mg/ml)	25.01 ± 3.97
MDML (6 mg/ml)	23.44 ± 3.32**
MDML (3 mg/ml)	9.38 ± 1.63
MDML (1.5 mg/ml)	7.81 ± 0.88
MDHL (10 mg/ml)	37.05 ± 2.69***
MDHL (8 mg/ml)	36.27 ± 1.70***
MDHL (6 mg/ml)	27.89 ± 2.18***
MDHL (3 mg/ml)	22.89 ± 1.79***
MDHL(1.5 mg/ml)	12.62 ± 1.89
MDTL (10 mg/ml)	15.95 ± 1.96*
MDTL(8 mg/ml)	13.62 ± 1.64*
MDTL (6 mg/ml)	12.28 ± 1.46*
MDTL (3 mg/ml)	10.49 ± 1.04*
MDTL (1.5 mg/ml)	9.89 ± 0.96*
MDCL(10 mg/ml)	29.81 ± 1.71***
MDCL (8 mg/ml)	21.17 ± 1.92**
MDCL (6 mg/ml)	15.61 ± 1.45**
MDCL (3 mg/ml)	12.32 ± 0.89***
MDCL (1.5 mg/ml)	9.52 ± 1.03*
MDEL (10 mg/ml)	16.15 ± 1.36**
MDEL (8 mg/ml)	8.49 ± 1.41
MDEL (6 mg/ml)	7.85 ± 0.73
MDEL (3 mg/ml)	6.26 ± 0.93
MDEL (1.5 mg/ml)	3.52 ± 0.90
MDMS (10 mg/ml)	42.21 ± 1.84***
MDMS (8 mg/ml)	29.27 ± 2.71**
MDMS (6 mg/ml)	26.89 ± 2.73**
MDMS (3 mg/ml)	18.40 ± 1.66**
MDMS (1.5 mg/ml)	17.89 ± 2.36*

The results were expressed in Mean ± SEM (standard mean error) *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ were considered as statistically significant. The statistical analysis followed by one-way analysis of variance (Dunnett's test) compared to the control. MDML = Crude methanol extract of *M. dispermus* leaves, MDHL = n-hexane fraction of crude methanol extract of *M. dispermus* leaves, MDTL = Carbon tetrachloride fraction of crude methanol extract of *M. dispermus* leaves, MDCL = Chloroform fraction of crude methanol extract of *M. dispermus* leaves, MDEL = Ethyl acetate fraction of crude methanol extract of *M. dispermus* leaves, MDMS = Crude methanol extract of *M. dispermus* stem barks

In vivo studies of *Macropanax dispermus* extracts

Evaluation of analgesic activity

Acetic acid-induced writhing method In this method, the investigated extracts at the doses of 200 and 400 mg/kg body weight showed a decrease in the number of writhing in rodents as compared to control. Among them, MDML and its MDCL, MDEL, MDTL fractions, and MDMS pro-

Table 3 Cytotoxic activity of the multiple extracts of *M. dispermus* leaves and stem barks

Sample	Equation	R^2	LC_{50} (µg/ml)
Standard (vincristine sulfate)	$y = 4.978x + 49.32$	$R^2 = 0.820$	0.14
MDML	$y = 0.028x + 2.916$	$R^2 = 0.914$	1681.57
MDHL	$y = 0.021x + 4.166$	$R^2 = 0.806$	2579.33
MDTL	$y = 0.018x + 5$	$R^2 = 0.677$	2500.00
MDCL	$y = 0.054x + 48.75$	$R^2 = 0.874$	23.15
MDEL	$y = 0.059x + 45$	$R^2 = 0.898$	84.75
MDAL	$y = 0.082x + 22.08$	$R^2 = 0.957$	340.49
MDMS	$y = 0.045x + 48.33$	$R^2 = 0.915$	37.11

The results were expressed in Mean ± SEM (standard mean error) *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ were considered as statistically significant. The statistical analysis followed by one-way analysis of variance (Dunnett's test) compared to the control. MDML = Crude methanol extract of *M. dispermus* leaves, MDHL = n-hexane fraction of crude methanol extract of *M. dispermus* leaves, MDTL = Carbon tetrachloride fraction of crude methanol extract of *M. dispermus* leaves, MDCL = Chloroform fraction of crude methanol extract of *M. dispermus* leaves, MDEL = Ethyl acetate fraction of crude methanol extract of *M. dispermus* leaves, MDAL = Aqueous fraction of crude methanol extract of *M. dispermus* leaves, MDMS = Crude methanol extract of *M. dispermus* stem barks

Table 4 Analgesic effect of the investigated extracts of M. dispermus leaves and stem barks on acetic acid-induced writhing test

Groups	No. of writhing	Inhibition of writhing (%)
Control	48.40 ± 1.44	–
Diclofenac Sodium (50 mg/kg)	4.20 ± 0.86***	91.32
MDML (200 mg/kg)	11.80 ± 1.43***	75.62
MDML (400 mg/kg)	7.60 ± 1.03***	84.30
MDHL (200 mg/kg)	19.00 ± 1.22***	60.74
MDHL (400 mg/kg)	11.20 ± 0.86***	76.86
MDTL (200 mg/kg)	18.00 ± 1.38***	62.81
MDTL (400 mg/kg)	10.00 ± 1.64***	79.34
MDCL (200 mg/kg)	14.20 ± 1.39***	70.66
MDCL (400 mg/kg)	8.60 ± 0.68***	82.23
MDEL (200 mg/kg)	11.40 ± 1.72***	76.45
MDEL (400 mg/kg)	9.60 ± 0.93***	80.17
MDAL (200 mg/kg)	22.20 ± 2.82***	54.13
MDAL (400 mg/kg)	13.20 ± 2.52***	72.73
MDMS (200 mg/kg)	14.40 ± 1.63***	70.25
MDMS (400 mg/kg)	11.20 ± 1.24***	76.86

The results were expressed in Mean ± SEM (standard mean error) *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ were considered as statistically significant. The statistical analysis followed by one-way analysis of variance (Dunnett's test) compared to the control. MDML = Crude methanol extract of *M. dispermus* leaves, MDHL = n-hexane fraction of crude methanol extract of *M. dispermus* leaves, MDTL = Carbon tetrachloride fraction of crude methanol extract of *M. dispermus* leaves, MDCL = Chloroform fraction of crude methanol extract of *M. dispermus* leaves, MDEL = Ethyl acetate fraction of crude methanol extract of *M. dispermus* leaves, MDAL = Aqueous fraction of crude methanol extract of *M. dispermus* leaves, MDMS = Crude methanol extract of *M. dispermus* stem barks

duced a significant ($p < 0.001$) decrease in the number of writhing (i.e., % inhibition of writhing) which demonstrated their higher effectiveness as peripheral analgesic agents as revealed in Table 4. Furthermore, MDML and its MDEL fraction comparatively showed the highest percentage of inhibition of writhing observed with both doses, 200 and 400 mg/kg relative to others.

Tail immersion method The extracts at doses of 200 and 400 mg/kg body weight showed a different level of increase in pain reaction time (PRT), percentage of maximal possible effect (%MPE), and percentage elongation of latency as compared to control in a dose-dependent manner. The 200 and 400 mg/kg doses of MDML, MDEL, MDAL, and MDMS showed significant ($p < 0.001$) analgesic activity. The same extracts exhibited a significant increase in PRT, higher %MPE, and percentage elongation of latency until 120 min. The %MPE of these extracts remained elevated throughout the observation period. In contrast, the standard morphine sulfate (10 mg/kg) increased the level significantly. MDML, MDEL, and MDMS demonstrated higher analgesic effects in both the acetic acid-induced writhing method and tail immersion method which indicated these extracts might show analgesic effect both peripherally and centrally. Among those, crude MDML and MDMS exhibited the highest effect in both methods. The results of PRT and %MPE are shown in Table 5, and the percentages of elongation of latency are presented in Table 6.

Formalin-induced paw licking method In this method, the experimental extracts (200 and 400 mg/kg body weight) showed a greater percentage (%) of pain inhibition in the late phase than that of the early phase of investigation as compared to control in mice significantly ($p < 0.001$). They exhibited their potential to give the analgesic effect peripherally more than centrally. Crude extracts MDML and MDMS showed significant peripheral and central analgesic effects which are observed with both doses (200 and 400 mg/kg). However, MDTL, MDCL, MDEL fractions of MDML extract produced significant ($p < 0.001$) analgesia peripherally. These results were tabulated in Table 7.

Evaluation of antipyretic activity

In brewer's yeast-induced pyrexia method, MDML and its related MDHL and MDEL fractions significantly reduced the rectal temperature ($p < 0.001$) after the 1 h of extract administration at the doses of 200 and 400 mg/kg body weight. All other investigated extracts (200 and 400 mg/kg) reduced the rectal temperature of Swiss albino mice as compared to control significantly ($p < 0.001$) on total (4 h) experimental period as displayed in Table 8. The MDTL, MDEL, MDHL fractions of MDML showed an increased reduction of rectal temperature. The percent decrease in

Table 5 Analgesic effect of the investigated extracts of *M. dispermus* leaves and stem barks on tail immersion test

Group	Pain reaction time in seconds (% MPE)				
	Pretreatment	30 min	60 min	90 min	120 min
Control	3.57 ± 0.43	4.69 ± 0.35 (9.77%)	4.32 ± 0.35 (6.58%)	4.65 ± 0.40 (9.42%)	4.14 ± 0.34 (5.01%)
Morphine sulfate (10 mg/kg)	3.96 ± 0.30	11.06 ± 0.35*** (64.31%)	12.52 ± 0.44*** (77.51%)	14.24 ± 0.57*** (93.15%)	14.36 ± 0.17*** (94.18%)
MDML (200 mg/kg)	3.88 ± 0.33	5.65 ± 0.22 (15.90%)	8.95 ± 0.33*** (45.62%)	10.08 ± 0.47*** (55.78%)	13.07 ± 0.42*** (82.66%)
MDML (400 mg/kg)	3.54 ± 0.46	7.95 ± 0.46*** (38.47%)	10.31 ± 0.38*** (59.10%)	13.07 ± 0.64*** (83.12%)	13.77 ± 0.50*** (89.28%)
MDHL (200 mg/kg)	3.92 ± 0.49	8.08 ± 0.52*** (37.52%)	8.77 ± 0.62*** (43.76%)	8.40 ± 0.43*** (40.44%)	7.10 ± 0.37** (28.75%)
MDHL (400 mg/kg)	3.87 ± 0.48	10.83 ± 0.59*** (62.52%)	10.98 ± 0.58*** (63.89%)	12.01 ± 0.73*** (73.12%)	8.48 ± 0.31*** (41.47%)
MDTL (200 mg/kg)	3.75 ± 0.22	5.57 ± 0.23 (16.14%)	7.59 ± 0.22** (34.13%)	9.83 ± 0.68*** (54.03%)	6.83 ± 0.32** (27.41%)
MDTL (400 mg/kg)	4.19 ± 0.25	7.99 ± 0.55*** (35.13%)	9.88 ± 0.52*** (52.63%)	12.15 ± 0.83*** (73.66%)	8.83 ± 0.53*** (42.93%)
MDCL (200 mg/kg)	3.86 ± 0.17	5.25 ± 0.30 (12.51%)	6.94 ± 0.66* (27.66%)	8.18 ± 0.30** (38.77%)	7.72 ± 0.53*** (34.69%)
MDCL (400 mg/kg)	4.34 ± 0.24	6.12 ± 0.20 (16.77%)	8.65 ± 0.59*** (40.49%)	10.09 ± 0.79*** (53.94%)	9.31 ± 0.32*** (46.62%)
MDEL (200 mg/kg)	3.88 ± 0.30	5.24 ± 0.24 (12.28%)	7.28 ± 0.20** (30.57%)	7.31 ± 0.38* (30.88%)	7.99 ± 0.37*** (37.04%)
MDEL (400 mg/kg)	4.63 ± 0.30	8.06 ± 0.44*** (33.05%)	9.15 ± 0.63*** (43.6%)	9.87 ± 0.40*** (50.55%)	10.43 ± 0.28*** (55.95%)
MDAL (200 mg/kg)	3.38 ± 0.80	5.72 ± 0.36 (20.12%)	9.62 ± 0.44*** (53.73%)	10.89 ± 0.39*** (64.62%)	12.62 ± 0.90*** (78.49%)
MDAL (400 mg/kg)	3.42 ± 0.18	6.71 ± 0.35** (28.42%)	11.03 ± 0.72*** (65.76%)	13.58 ± 0.43*** (87.71%)	13.79 ± 0.72*** (89.52%)
MDMS (200 mg/kg)	4.09 ± 0.38	6.43 ± 0.34 (21.43%)	8.77 ± 0.82*** (42.85%)	7.06 ± 0.63* (27.21%)	11.80 ± 0.55*** (70.65%)
MDMS (400 mg/kg)	4.09 ± 0.25	9.11 ± 0.44*** (45.95%)	10.89 ± 0.62*** (62.26%)	11.27 ± 0.61*** (65.79%)	13.88 ± 0.75*** (89.69%)

The results were expressed in Mean ± SEM (standard mean error) *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ were considered as statistically significant. The statistical analysis followed by one-way analysis of variance (Dunnett's test) compared to the control. MDML = Crude methanol extract of *M. dispermus* leaves, MDHL = n-hexane fraction of crude methanol extract of *M. dispermus* leaves, MDTL = Carbon tetrachloride fraction of crude methanol extract of *M. dispermus* leaves, MDCL = Chloroform fraction of crude methanol extract of *M. dispermus* leaves, MDEL = Ethyl acetate fraction of crude methanol extract of *M. dispermus* leaves, MDAL = Aqueous fraction of crude methanol extract of *M. dispermus* leaves, MDMS = Crude methanol extract of *M. dispermus* stem barks

Table 6 Analgesic activity of the investigated extracts of *M. dispermus* leaves and stem barks on tail immersion test

Group	Dose (mg/kg)	Percent (%) elongation of latency			
		30 min	60 min	90 min	120 min
Morphine sulfate	10	57.61	65.46	67.37	71.13
MDML 200	200	17.03	51.71	53.91	68.30
MDML 400	400	41.03	58.07	64.43	69.91
MDHL 200	200	41.95	50.68	44.67	41.67
MDHL 400	400	56.70	60.62	61.29	51.16
MDTL 200	200	15.77	43.03	52.71	39.36
MDTL 400	400	41.29	56.23	61.75	53.07
MDCL 200	200	10.70	37.68	43.15	46.34
MDCL 200	400	23.45	50.03	53.93	55.48
MDEL 400	200	10.60	40.59	36.43	48.19
MDEL 200	400	41.84	56.76	52.93	60.28
MDAL 400	200	17.98	55.06	57.31	67.15
MDAL 200	400	30.13	60.81	65.76	69.94
MDMS 200	200	27.14	50.70	34.20	64.88
MDMS 400	400	48.53	60.28	58.76	70.14

The results were expressed in Mean ± SEM (standard mean error) $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ were considered as statistically significant. The statistical analysis followed by one-way analysis of variance (Dunnett's test) compared to the control. MDML = Crude methanol extract of *M. dispermus* leaves, MDHL = n-hexane fraction of crude methanol extract of *M. dispermus* leaves, MDTL = Carbon tetrachloride fraction of crude methanol extract of *M. dispermus* leaves, MDCL = Chloroform fraction of crude methanol extract of *M. dispermus* leaves, MDEL = Ethyl acetate fraction of crude methanol extract of *M. dispermus* leaves, MDAL = Aqueous fraction of crude methanol extract of *M. dispermus* leaves, MDMS = Crude methanol extract of *M. dispermus* stem barks

rectal temperature produced by these extracts conferred in Table 9.

Discussion

The preliminary phytochemical analysis showed that crude methanol extract of leaves, its solvent fractions, and crude methanol extracts of stem barks of *M. dispermus* contain a mixture of phytochemical compounds that had potential biological and therapeutic activities [43, 44]. Therefore this species was expected to have many medicinal uses. The present research on this plant showed that the crude MDML and MDMS along with some fractions possess considerable thrombolytic, cytotoxic, analgesic, and antipyretic properties.

Several types of research have been conducted to find antithrombotic agents from different types of medicinal plants. From the current study, it was evident that the clot dissolution did not occur after the addition of distilled water to the clot. In contrast, standard streptokinase lysed almost all the clots. During the investigation, it was observed that MDHL and MDMS were observed to have a significant thrombolytic effect. Among those, MDHL showed a significant thrombolytic effect with no potential cytotoxic effect. The clot lysis effect of these investigated extracts increased with the increase in their concentration. Since the thrombolytic effect of those extracts was comparable to that of Streptokinase, they possibly acted through the formation of a complex with plasminogen which could convert additional plasminogen to plasmin. Plasmin lysed clots by breaking down the fibrinogen and fibrin contained in a clot [45]. Although the role of alkaloids, saponins, tannins, terpenoids, and flavonoids as thrombolytic agents is still unknown, their presence in those extracts might contribute to thrombolysis through platelet aggregation [46, 47].

In the cytotoxicity evaluation study of plant extracts by brine shrimp lethality assay, LC_{50} values lower than 1000 µg/ml were considered cytotoxic [42]. From the current observation, it was found that vincristine sulfate, a known anticancer agent had the lowest LC_{50} value of 0.14 µg/ml which proved its highly potent cytotoxic activity. In this study, MDML extract and its MDHL and MDTL fractions were evident as a highly non-cytotoxic agent. But MDCL, MDEL, MDAL fractions of crude MDML, and MDMS were evident to possess cytotoxic activity. Hence they could be further investigated for their potential as anticancer agents. Preliminary phytochemical screening revealed the presence of flavonoids, glycosides, saponin, alkaloids, and steroids in the investigated extracts which might contribute to their cytotoxic activities [48, 49]. Flavonoids exert their cytotoxic activity by increasing the production of cellular ROS levels [50]. Saponins initiate apoptosis of cancer cells [51]. Previous studies suggested that alkaloids can inhibit the proliferation of many cancer cells [52].

The acetic acid-induced writhing method was used to evaluate the peripheral analgesic activity of the investigated extracts which used the reaction of animals to painful chemical stimuli [53, 54]. In this method, dilute acetic acid produced a writhing reflex in animals by activating the chemosensitive nociceptors [55]. It produced a localized inflammatory response due to the release of free Arachidonic acid from tissue phospholipid via COX and produced prostaglandins in the peritoneal fluid. It causes swelling and the release of endogenous substances that stimulate pain nerve endings [56]. The crude MDML, and its MDTL, MDCL, and MDEL fractions at the dose of 200 and 400 mg/kg showed a significant and higher percentage (%) of inhibition of abdominal writhing in mice as compared to control whereas the standard Diclofenac sodium, an NSAID at 50 mg/kg showed the highest activity. The significant ($P < 0.001$) dose-dependent analgesic effect of the investigated extracts was hypothesized to be mediated through peripheral pain mechanism and suppression of prostaglandin pathway as they had a comparable analgesic effect like NSAID [57].

Table 7 Analgesic effect of the investigated extracts of *M. dispermus* leaves and stem barks on formalin-induced paw licking test

Group	0–5 min (early phase)		15–30 min (late phase)	
	Paw licking time (s)	Percent inhibition of pain	Paw licking time (s)	Percent Inhibition of PAIN
Control	52.98 ± 4.73	–	72.76 ± 5.55	–
Morphine sulfate (10 mg/kg)	8.71 ± 0.66***	83.55	4.90 ± 0.66***	93.27
MDML (200 mg/kg)	23.72 ± 3.43**	55.23	18.49 ± 1.23***	74.59
MDML (400 mg/kg)	21.51 ± 3.83**	59.40	12.57 ± 1.95***	82.72
MDHL (200 mg/kg)	49.61 ± 8.07	6.37	43.65 ± 2.25***	40.02
MDHL (400 mg/kg)	37.96 ± 6.82	28.35	18.27 ± 3.42***	74.89
MDTL (200 mg/kg)	41.76 ± 3.63	21.18	28.17 ± 4.74***	61.29
MDTL (400 mg/kg)	34.54 ± 6.81	34.81	12.20 ± 2.13***	83.23
MDCL (200 mg/kg)	49.76 ± 5.05	6.08	29.70 ± 3.61***	59.18
MDCL (400 mg/kg)	32.68 ± 4.05	38.31	25.43 ± 4.37***	65.05
MDEL (200 mg/kg)	52.36 ± 6.69	1.17	14.98 ± 2.68***	79.41
MDEL (400 mg/kg)	33.17 ± 4.87	37.39	8.87 ± 1.06***	87.80
MDAL (200 mg/kg)	46.85 ± 5.20	11.58	63.95 ± 4.91	12.12
MDAL (400 mg/kg)	29.71 ± 4.42*	43.93	19.72 ± 3.08***	72.90
MDMS (200 mg/kg)	45.97 ± 4.52	13.23	16.65 ± 2.26***	77.12
MDMS (400 mg/kg)	24.21 ± 2.34**	54.30	7.56 ± 0.62***	89.61

The results were expressed in Mean ± SEM (standard mean error) *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ were considered as statistically significant. The statistical analysis followed by one-way analysis of variance (Dunnett's test) compared to the control. MDML = Crude methanol extract of *M. dispermus* leaves, MDHL = n-hexane fraction of crude methanol extract of *M. dispermus* leaves, MDTL = Carbon tetrachloride fraction of crude methanol extract of *M. dispermus* leaves, MDCL = Chloroform fraction of crude methanol extract of *M. dispermus* leaves, MDEL = Ethyl acetate fraction of crude methanol extract of *M. dispermus* leaves, MDAL = Aqueous fraction of crude methanol extract of *M. dispermus* leaves, MDMS = Crude methanol extract of *M. dispermus* stem barks

Table 8 Antipyretic effect of the investigated extracts of *M. dispermus* leaves and stem barks on brewer's yeast-induced pyrexia test

Group	Normal rectal temperature (°F)	After yeast administration	Rectal temperature after drug administration (°F)			
			1 h	2 h	3 h	4 h
Control	97.34 ± 0.7	100.96 ± 0.32	101.72 ± 0.37	102.40 ± 0.30	102.42 ± 0.34	102.12 ± 0.35
Paracetamol (150 mg/kg)	96.82 ± 0.62	100.48 ± 0.27	98.08 ± 0.32***	97.48 ± 0.34***	97.12 ± 0.47***	96.82 ± 0.53***
MDML (200 mg/kg)	97.02 ± 0.66	99.82 ± 0.10	98.96 ± 0.28***	98.10 ± 0.38***	97.62 ± 0.41***	97.84 ± 0.40***
MDML (400 mg/kg)	98.50 ± 0.42	100.06 ± 0.42	99.54 ± 0.19***	98.96 ± 0.28***	98.68 ± 0.31***	98.88 ± 0.26***
MDHL (200 mg/kg)	97.22 ± 0.71	100.76 ± 0.42	100.30 ± 0.49***	99.90 ± 0.47***	98.90 ± 0.38***	98.30 ± 0.33***
MDHL (400 mg/kg)	98.06 ± 0.30	100.34 ± 0.42	99.66 ± 0.48***	98.76 ± 0.41***	98.30 ± 0.30***	98.16 ± 0.33***
MDTL (200 mg/kg)	98.60 ± 0.20	101.06 ± 0.19	101.48 ± 0.15	100.64 ± 0.14**	99.14 ± 0.34***	99.30 ± 0.32***
MDTL (400 mg/kg)	97.58 ± 0.47	99.98 ± 0.13	100.88 ± 0.35	99.24 ± 0.35***	98.08 ± 0.29***	97.62 ± 0.34***
MDCL (200 mg/kg)	97.88 ± 0.48	101.28 ± 0.21	101.42 ± 0.24	101.62 ± 0.26	99.58 ± 0.27***	98.74 ± 0.19***
MDCL (400 mg/kg)	97.10 ± 0.37	100.86 ± 0.27	101.08 ± 0.30	101.48 ± 0.40	98.80 ± 0.28***	97.82 ± 0.10***
MDEL (200 mg/kg)	97.02 ± 0.55	100.92 ± 0.37	99.80 ± 0.28**	99.18 ± 0.26***	98.44 ± 0.18***	98.16 ± 0.15***
MDEL (400 mg/kg)	97.32 ± 0.30	100.46 ± 0.18	99.32 ± 0.13***	98.50 ± 0.29***	97.64 ± 0.37***	97.42 ± 0.28***
MDAL (200 mg/kg)	96.68 ± 0.33	100.52 ± 0.29	100.66 ± 0.32	101.16 ± 0.3	98.88 ± 0.38***	98.74 ± 0.37***
MDAL (400 mg/kg)	97.38 ± 0.31	101.28 ± 0.42	101.54 ± 0.43	100.42 ± 0.37**	98.80 ± 0.34***	98.34 ± 0.20***
MDMS (200 mg/kg)	97.66 ± 0.65	100.08 ± 0.15	101.00 ± 0.28	99.92 ± 0.23***	98.62 ± 0.44***	98.72 ± 0.40***
MDMS (400 mg/kg)	96.64 ± 0.40	99.48 ± 0.44	100.32 ± 0.25*	98.90 ± 0.54***	97.30 ± 0.29***	97.10 ± 0.32***

The results were expressed in Mean ± SEM (standard mean error) *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ were considered as statistically significant. The statistical analysis followed by one-way analysis of variance (Dunnett's test) compared to the control. MDML = Crude methanol extract of *M. dispermus* leaves, MDHL = n-hexane fraction of crude methanol extract of *M. dispermus* leaves, MDTL = Carbon tetrachloride fraction of crude methanol extract of *M. dispermus* leaves, MDCL = Chloroform fraction of crude methanol extract of *M. dispermus* leaves, MDEL = Ethyl acetate fraction of crude methanol extract of *M. dispermus* leaves, MDAL = Aqueous fraction of crude methanol extract of *M. dispermus* leaves, MDMS = Crude methanol extract of *M. dispermus* stem barks

Table 9 Percent decrease in rectal temperature of all test samples of *M. dispermus* leaves and stem barks on brewer's yeast-induced pyrexia test

Group	Percent decrease in rectal temperature (%) after drug treatment			
	1 h	2 h	3 h	4 h
Paracetamol (150 mg/kg)	65.57	81.97	91.8	100
MDML (200 mg/kg)	30.71	61.43	78.57	70.71
MDML (400 mg/kg)	33.33	70.51	88.46	75.64
MDHL (200 mg/kg)	12.99	24.29	52.54	69.49
MDHL (400 mg/kg)	29.82	69.3	89.47	95.61
MDTL (200 mg/kg)	–	17.07	78.05	71.54
MDTL (400 mg/kg)	–	30.83	79.17	98.33
MDCL (200 mg/kg)	–	–	50.00	74.71
MDCL (400 mg/kg)	–	–	54.79	80.85
MDEL (200 mg/kg)	28.72	44.62	63.59	70.77
MDEL (400 mg/kg)	36.31	62.42	89.81	96.82
MDAL (200 mg/kg)	–	–	42.71	46.35
MDAL (400 mg/kg)	–	22.05	63.59	75.38
MDMS (200 mg/kg)	–	6.61	60.33	56.2
MDMS (400 mg/kg)	–	20.42	76.76	83.8

The results were expressed in Mean ± SEM (standard mean error) $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ were considered as statistically significant. The statistical analysis followed by one-way analysis of variance (Dunnett's test) compared to the control. MDML = Crude methanol extract of *M. dispermus* leaves, MDHL = *n*-hexane fraction of crude methanol extract of *M. dispermus* leaves, MDTL = Carbon tetrachloride fraction of crude methanol extract of *M. dispermus* leaves, MDCL = Chloroform fraction of crude methanol extract of *M. dispermus* leaves, MDEL = Ethyl acetate fraction of crude methanol extract of *M. dispermus* leaves, MDAL = Aqueous fraction of crude methanol extract of *M. dispermus* leaves, MDMS = Crude methanol extract of *M. dispermus* stem barks, '–' indicates the reduction of temperature didn't occur at a time interval

The central analgesic activity evaluation model of tail immersion used thermal stimuli in this regard [54, 58]. In this model, sensory nerves sensitized the nociceptors, increased the pain thresholds [59], and the involvement of prostaglandins was decreased [60]. In the current study, the increase in %MPE, PRT, or latency period indicated the level of analgesia of drug or extract. MDML, MDEL, MDAL, and MDMS at 200 and 400 mg/kg showed a significant increase in PRT, higher percentage (%) of MPE, and elongation until 120 min as compared to control in a dose-dependent manner whereas the reference standard morphine sulfate (10 mg/kg) also increased those indices significantly. Since their effect was comparable to narcotic drug morphine sulfate, those extracts possibly showed an opiates-like mechanism of action indicating the spinal and supraspinal pathways of analgesia [59].

The analgesic model of formalin-induced paw licking test usually involved two distinct phases: an early transient phase, which occurred in the first 5 min that reflected direct stimulation of nociceptors, and a late tonic phase, evident 15 to 30 min after injection that might be associated with the release of inflammatory mediators [61, 62]. In this method, MDML and MDMS showed significant inhibition of induced pain during both early and late phases of the study as compared to control. MDTL and MDEL fractions also showed highly significant effectiveness in pain inhibition during the late phase that demonstrated its dominance as a peripheral analgesic. Several reports had shown the analgesic properties of flavonoids, tannins, and other polyphenolic compounds in different experimental animal models [63]. The presence of those phytochemicals in the extracts could be attributed to their effectiveness as analgesic agents.

In the brewer's yeast-induced pyrexia model, the proteins present in yeast were responsible for fever induction through an inflammatory reaction [64]. In this experiment, n-hexane, carbon tetrachloride and ethyl acetate fractions of crude methanol extracts of *M. dispermus* leaves at the dose of 200 and 400 mg/kg exhibited an increasing percentage (%) of reduction of hyperthermia in a significant ($P < 0.001$) dose-dependent manner which persists up to 4 h. This effect was comparable to the standard drug paracetamol. Hence, there might be a possible mechanism of antipyretic action by inhibiting the synthesis of prostaglandins like paracetamol [65]. MDTL and MDEL exhibited both peripheral analgesic effect and antipyretic effect possibly showed action through inhibition of the synthesis of prostaglandins. So, they might act as cyclooxygenase inhibitors [66]. Furthermore, several multi-processes or mediators such as bacterial endotoxin (lipopolysaccharide), endogenous pyrogen, and interleukin-1α were emphasizing the pathogenesis of fever. Inhibition of any of these mediators might bring about antipyresis [67]. The antipyretic activity could be triggered by the presence of flavonoids, saponins, glycosides, tannins in the investigated extracts [68].

Conclusions

The current research found that the different extracts of *M. dispermus* leaves and stem barks revealed the presence of a mixture of phytochemicals and exhibited potent thrombolytic, anticancer, analgesic, and antipyretic effects. It may have concluded an important contribution in the herbal medicine researches and further advanced researches must be conducted on this medicinal plant to discover its more precise physiologic effect.

Abbreviations

DW: Distilled water; SK: Streptokinase; GABA: Gamma amino butyric acid; MPE: Maximal possible effect; PG: Prostaglandins; LC: Lethal concentration; PRT: Pain reaction time; NSAID: Non-steroidal anti-inflammatory drugs.

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Authors' contributions
MKH designed the experiments, conception, and supervised the research work. SRA collected the plant material, performed the pharmacological assays, statistical analysis, and drafted the manuscript. NMP, SSD, and SWJ contributed to performing pharmacological analysis. MRI helps in the statistical analysis and write-up of the manuscript. MRI and BHK critically revised the manuscript, provided punctual assistance, and gave the final approval for the submission of a revised version. Finally, all authors gave their final consent for the submission. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the ethical committee of the University of Chittagong, Bangladesh under the approval no-cc98056.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References

1. Sofowora A (1982) Medicinal plants and traditional medicine in Africa. Wiley
2. Mannan A, Kawser M, Ahmed A, Islam NN, Alam S, Emon MAEK, Gupta SD (2011) Assessment of antibacterial, thrombolytic and cytotoxic potential of *Cassia alata* seed oil. J Appl Pharm Sci 1(9):56
3. Collen D (1990) Coronary thrombolysis: streptokinase or recombinant tissue-type plasminogen activator? Ann Intern Med 112(7):529–538
4. Nicolini FA, Nichols WW, Mehta JL, Saldeen TG, Schofield R, Ross M, Player DW, Pohl GB, Mattsson C (1992) Sustained reflow in dogs with coronary thrombosis with K2P, a novel mutant of tissue-plasminogen activator. J Am Coll Cardiol 20(1):228–235
5. Rouf SA, Moo-Young M, Chisti Y (1996) Tissue-type plasminogen activator: characteristics, applications and production technology. Biotechnol Adv 14(3):239–266
6. Awadh N, Ronco JJ, Bernstein V, Gilks B, Wilcox P (1994) Spontaneous pulmonary hemorrhage after thrombolytic therapy for acute myocardial infarction. Chest 106(5):1622–1624
7. Cooper JP, Quarry DP, Beale DJ, Chappell AG (1994) Life-threatening, localized angio-oedema associated with streptokinase. Postgrad Med J 70(826):592
8. Rahman MA, Sultana R, Emran TB, Islam MS, Rahman MA, Chakma JS, Rashid H-u, Hasan CMM (2013) Effects of organic extracts of six Bangladeshi plants on in vitro thrombolysis and cytotoxicity. BMC Complement Altern Med 13(1):25
9. Uddin MS, Millat MS, Islam MS, Hussain MS, Uddin MG, Siddiqui SA, Ferdous M (2020) Exploration of in vitro thrombolytic, anthelminthic, cytotoxic and in vivo anxiolytic potentials with phytochemical screening of flowers of *Brassica nigra*. Futur J Pharm Sci 6(1):1–9
10. Schug SA, Daly HC, Stannard KJ (2011) Pathophysiology of pain. In: Fitridge R, Thompson M (eds) Mechanisms of vascular disease: A reference book for vascular specialists. University of Adelaide Press
11. Sen S, Chakraborty R, De B, Ganesh T, Raghavendra H, Debnath S (2010) Analgesic and anti-inflammatory herbs: a potential source of modern medicine. Int J Pharm Sci Res 1(11):32
12. Hewitt D, Hargreaves R, Curtis S, Michelson D (2009) Challenges in analgesic drug development. Clin Pharmacol Ther 86:447–450
13. Walter EJ, Hanna-Jumma S, Carraretto M, Forni L (2016) The pathophysiological basis and consequences of fever. Crit Care 20(1):1–10
14. Mate G, Naikwade N, Magdum C, Chowki A, Patil S (2008) Evaluation of anti-nociceptive activity of *Cissus quadrangularis* on albino mice. Int l J Green Pharma 2:118–121
15. Pan S-Y, Litscher G, Gao S-H, Zhou S-F, Yu Z-L, Chen H-Q, Zhang S-F, Tang M-K, Sun J-N, Ko K-M (2014) Historical perspective of traditional indigenous medical practices: the current renaissance and conservation of herbal resources. Evid-Based Complement Alternat Med. 2014:525340
16. Chopra RN, Nayar SL, Chopra IC (1956) Glossary of Indian medicinal plants, vol 1. Council of Scientific & Industrial Research
17. Phillipson JD (2001) Phytochemistry and medicinal plants. Phytochem 56(3):237–243
18. Cragg GM, Newman DJ (2005) Plants as a source of anti-cancer agents. J Ethnopharmacol 100(1–2):72–79
19. Rates SMK (2001) Plants as source of drugs. Toxicon 39(5):603–613
20. Queiroz EF, Wolfender J-L, Hostettmann K (2009) Modern approaches in the search for new lead antiparasitic compounds from higher plants. Curr Drug Targets 10(3):202–211
21. Panyaphu K, Van On T, Sirisa-Ard P, Srisa-Nga P, ChansaKaow S, Nathakarnkitkul S (2011) Medicinal plants of the Mien (Yao) in Northern Thailand and their potential value in the primary healthcare of postpartum women. J Ethnopharmacol 135(2):226–237
22. Ong HG, Ling SM, Win TTM, Kang D-H, Lee J-H, Kim Y-D (2018) Ethnobotany of wild medicinal plants used by the Müün ethnic people: a quantitative survey in southern Chin state. Myanmar J Herb Med 13:91–96
23. Chanwitheesuk A, Teerawutgulrag A, Rakariyatham N (2005) Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. Food Chem 92:491–497
24. Kupchan SM, Tsou G, Sigel CW (1973) Datiscacin, a novel cytotoxic cucurbitacin 20-acetate from Datisca glomerata. J Org Chem 38(7):1420–1421
25. VanWagenen BC, Larsen R, Cardellina JH, Randazzo D, Lidert ZC, Swithenbank C (1993) Ulosantoin, a potent insecticide from the sponge Ulosa ruetzleri. J Org Chem 58(2):335–337
26. Sofowora A (1996) Research on medicinal plants and traditional medicine in Africa. J Altern Complement Med 2(3):365–372
27. Trease G, Evans W (1989) Pharmacognosy. Bailliere Tindall Ltd
28. Harborne J (1973) Phytochemicals methods. Chapman and Hall Ltd
29. Al-Araby S, Rahman MA, Chowdhury MA, Das R, Chowdhury T, Hasan CMM, Afroze M, Hashem M, Hajjar D, Alelwani W (2020) *Padina tenuis* (marine alga) attenuates oxidative stress and streptozotocin-induced type 2 diabetic indices in Wistar albino rats. S Afr J Bot 128:87–100
30. Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM, Daginawala HF (2006) Development of an in vitro model to study clot lysis activity of thrombolytic drugs. Thrombosis J 4(1):14
31. Mosaddik MA, Haque ME (2003) Cytotoxicity and antimicrobial activity of goniothalamin isolated from *Bryonopsis laciniosa*. Phytother Res 17(10):1155–1157
32. Sathasivam P, Lakshmi T (2017) Brine shrimp lethality assay of *Sesbania grandiflora* ethanolic extract—in vitro study. J Adv Pharm Edu Res 7(1):28–30
33. Koster R, Anderson M, de Beer EJ (1959) Acetic acid for analgesic screening. In: Fed Proc, p 412

34. Dambisya YM, Lee T-L, Sathivulu V, Jais AMM (1999) Influence of temperature, pH and naloxone on the antinociceptive activity of *Channa striatus* (haruan) extracts in mice. J Ethnopharmacol 66(2):181–186
35. Di Stasi LC, Costa M, Mendaçolli SL, Kirizawa M, Gomes C, Trolin G (1988) Screening in mice of some medicinal plants used for analgesic purposes in the state of Sao Paulo. J Ethnopharmacol 24:205–211
36. Malairajan P, Gopalakrishnan G, Narasimhan S, Veni KJK (2006) Analgesic activity of some Indian medicinal plants. J Ethnopharmacol 106(3):425–428
37. Fan S-H, Ali NA, Basri DF (2014) Evaluation of analgesic activity of the methanol extract from the galls of *Quercus infectoria* (Olivier) in rats. Evid-Based Complement Alternat Med. https://doi.org/10.1155/2014/976764
38. Kumawat RK, Kumar S, Sharma S (2012) Evaluation of analgesic activity of various extracts of *Sida tiagii* Bhandari. Acta Pol Pharm 69:1103–1109
39. Hunskaar S, Hole K (1987) The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 30:103–114
40. Adams S, Hebborn P, Nicholson J (1968) Some aspects of the pharmacology of ibufenac, a non-steroidal anti-inflammatory agent. J Pharm Pharmacol 20:305–312
41. Muhammad N, Saeed M, Khan H (2012) Antipyretic, analgesic and anti-inflammatory activity of *Viola betonicifolia* whole plant. BMC Complement Altern Med 12:59
42. Meyer B, Ferrigni N, Putnam J, Jacobsen L, Dj N, McLaughlin JL (1982) Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med 45(05):31–34
43. Benedec D, Vlase L, Oniga I, Mot A, Damian G, Hanganu D, Duma M, Silaghi-Dumitrescu R (2013) Polyphenolic composition, antioxidant and antibacterial activities for two Romanian subspecies of *Achillea distans* Waldst. et Kit. ex Willd. Molecules 18(8):8725–8739
44. Rao BG, Rao ES, Rao TM (2012) Quantification of phytochemical constituents and in-vitro antioxidant activity of *Mesua ferrea l*eaves. Asian Pac J Trop Biomed 2(2):S539–S542
45. Banerjee A, Chisti Y, Banerjee U (2004) Streptokinase—a clinically useful thrombolytic agent. Biotechnol Adv 22(4):287–307
46. Islam A, Hussain MS, Sen N, Abedin F, Millat MS, Islam MS, Das A, Kar A, Hossain M (2017) Investigation of in vitro thrombolytic and anti-helminthic activity and in vivo anxiolytic and antidepressant potentiality with phytochemical nature of methanolic extract of *Leucas lavandulifolia*. Sustain Chem Pharm 6:61–66
47. Klafke JZ, Arnoldi da Silva M, Fortes Rossato M, Trevisan G, Banderó Walker CI, Martins Leal CA, Olschowsky Borges D, Chitolina Schetinger MR, Noal Moresco R, Medeiros Frescura Duarte MM et al (2012) Antiplatelet, antithrombotic, and fibrinolytic activities of *Campomanesia xanthocarpa*. Evid-Based Complement Alternat Med 2012:954748
48. Dhar M, Dhar M, Dhawan B, Mehrotra B, Srimal R, Tandon J (1973) Screening of Indian plants for biological activity. IV. Indian J Exp Biol 11(1):43–54
49. Badami S, Manohara Reddy S, Kumar E, Vijayan P, Suresh B (2003) Antitumor activity of total alkaloid fraction of *Solanum pseudocapsicum* leaves. Phytother Res 17(9):1001–1004
50. Matsuo M, Sasaki N, Saga K, Kaneko T (2005) Cytotoxicity of flavonoids toward cultured normal human cells. Biol Pharm Bull 28(2):253–259
51. Alam F, us Saqib QN, Waheed A (2017) Cytotoxic activity of extracts and crude saponins from *Zanthoxylum armatum* DC. against human breast (MCF-7, MDA-MB-468) and colorectal (Caco-2) cancer cell lines. BMC Complement Altern Med 17(1):368
52. Lamchouri F, Zemzami M, Jossang A, Abdellatif A, Israili ZH, Lyoussi B (2013) Cytotoxicity of alkaloids isolated from *Peganum harmala* seeds. Pak J Pharm Sci 26(4):699–706
53. Hasan SR, Hossain M, Akter R, Jamila M, Mazumder M, Alam M, Faruque A, Rana S, Rahman S (2010) Analgesic activity of the different fractions of the aerial parts of *Commelina benghalensis* Linn. Int J Pharmacol 6(1):63–67
54. George K, Eric W, David D, George A (2009) Antinociceptive effects of *Newboulia laveis* (P. Beauv) stem bark extract in rat model. Pharmacog Mag 17:49–54
55. Ezeja M, Omeh Y, Ezeigbo I, Ekechukwu A (2011) Evaluation of the analgesic activity of the methanolic stem bark extract of *Dialium guineense* (Wild). Ann Med Health Sci Res 1:55–62
56. Afsar T, Khan MR, Razak S, Ullah S, Mirza B (2015) Antipyretic, anti-inflammatory and analgesic activity of *Acacia hydaspica* R. Parker and its phytochemical analysis. BMC Complement Altern Med 15(1):1–12
57. Ferdous M, Rouf R, Shilpi JA, Uddin SJ (2008) Antinociceptive activity of the ethanolic extract of *Ficus racemosa* Lin. (Moraceae). Orient Pharma Exp Med 8:93–96
58. Björkman R (1995) Central antinociceptive effects of non-steroidal anti-inflammatory drugs and paracetamol. Experimental studies in the rat. Acta Anaesthesiol Scand Suppl 103:1–44
59. Shah SMM, Shah SMH (2015) Phytochemicals, antioxidant, antinociceptive and anti-inflammatory potential of the aqueous extract of *Teucrium stocksianum* bioss. BMC Complement Altern Med 15(1):1–7
60. Bachhav R, Gulecha V, Upasani C (2009) Analgesic and anti-inflammatory activity of *Argyreia speciosa* root. Ind J Pharmacol 41:158
61. Dubuisson D, Dennis SG (1977) The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain 4:161–174
62. Rosland JH, Tjølsen A, Mæhle B, Hole K (1990) The formalin test in mice: effect of formalin concentration. Pain 42(2):235–242
63. Krasteva I, Platikanov S, Nikolov S, Kaloga M (2007) Flavonoids from *Astragalus hamosus*. Nat Prod Res 21(5):392–395
64. Pasin JSM, Ferreira APO, Saraiva ALL, Ratzlaff V, Andrighetto R, Tomazetti J et al (2010) Diacerein decreases TNF-α and IL-1β levels in peritoneal fluid and prevents Baker's yeast-induced fever in young rats. Inflam Res 59:189–196
65. Chandrasekharan N, Dai H, Roos KLT, Evanson NK, Tomsik J, Elton TS et al (2002) COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. Proc Natl Acad Sci 99:13926–13931
66. Yemitan OK, Adeyemi OO (2017) Mechanistic assessment of the analgesic, anti-inflammatory and antipyretic actions of *Dalbergia saxatilis* in animal models. Pharm Biol 55(1):898–905
67. Srivastava S, Singh P, Jha K, Mishra G, Srivastava S, Khosa R (2013) Antiinflammatory, analgesic and antipyretic activities of aerial parts of *Costus speciosus* Koen. Ind J Pharma Sci 75:83
68. Mutalik S, Paridhavi K, Rao CM, Udupa N (2003) Antipyretic and analgesic effect of leaves of *Solanum melongena* Linn. in rodents. Indian J Pharmacol 35(5):312–315

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