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ETHNOMEDICINAL, PHYTOCHEMICAL, AND PHARMACOLOGICAL PROFILE OF *MICROCOS PANICULATA* L.

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ABSTRACT

Microcos paniculata L., a medicinal plant belonging to the Tiliaceae family, has long been used in traditional medicine across Asia to manage ailments such as diarrhea, fever, wounds, and various gastrointestinal and inflammatory disorders. This review presents an overview of the plant's phytochemical profile and pharmacological potential, with a particular emphasis on its rich flavonoid content and multifaceted bioactivity. A total of 70 active constituents—including flavonoids, alkaloids, terpenoids, organic acids, and steroids—have been identified from its leaves, fruits, barks, and roots. Scientific investigations highlight its significant biological activities, including pain-relieving, anti-inflammatory, antimicrobial, cytotoxic, antioxidant, antidiabetic, membrane-protective, and thrombolytic effects. These therapeutic actions have been confirmed through various experimental models such as the formalin-induced paw licking test, hot plate and tail immersion methods, gastrointestinal motility studies, antidiarrheal evaluations, and brine shrimp lethality assays. Due to its broad pharmacological scope and chemical complexity, M. paniculata is a strong candidate for the development of herbal drugs and functional food products. However, additional research is necessary to clarify its mechanisms of action, safety parameters, and clinical applicability.

KEYWORDS: Microcos paniculata, bioactive compounds, flavonoids, analgesic activity, anti-inflammatory, antidiarrheal, antimicrobial potential, cytotoxicity, thrombolysis, ethnomedicine.

INTRODUCTION

Plants provide complicated, mixed, and distinct nonnutrient elements which act as the main basis of drug discovery.[1] Plant extracts contain phytochemical constituents for miscellaneous medicinal activities which are bioactive in nature.[2] The genus Microcos, belonging to the family Tiliaceae, comprises 60 species worldwide, spreading across African countries, India, Malaysia and Indonesia. In China, there are three species, including Microcos paniculata L., Microcos chungii (merr.) chun and Microcos stauntoniana G. don, which are mainly found across Southwest and West China.[3] M. paniculata, also known as Grewia nervosa, is a medicinal and edible plant.[4] These plants being shrubs or small trees. The dried bark, roots, fruits, and leaves of Microcos species have been used ethnomedicially to treat diarrhea and fever, as general tonics, and as insecticides.[5] Kathgua or Fattashi is the local name of Microcos paniculata (family: Tiliaceae) in Bangladesh that are harvested all over Bangladesh. Generally it develops naturally as a shrub or short tree. This plant is known for many traditional uses, for example, to treat diarrhea, wounds, cold, fever, hepatitis, dyspepsia, and heat stroke. Moreover it has insecticidal activity. However, it is active against the digestive system also. Thorough study of literature revealed that it showed several activities, including analgesic, antimicrobial, neuropharmacological, α-glucosidase inhibition, brine shrimp lethality, free radical scavenging, antipyretic, nicotinic receptor antagonistic, larvicidal, cytotoxic, insecticidal, anti-inflammatory, and antidiarrheal activities. In addition, it can prevent angina pectoris, coronary heart disease, or coronary artery disease or ischaemic heart disease. Acute toxicity study was also carried out.[6-11]

In the current review, we aim at summarizing the traditional uses, botany, phytochemicals, pharmacological activities, quality control and toxicology of MPL leaves, fruits, barks and roots extracts reported over the past decades. These up-to-date research observations will be helpful in understanding the biological activities of this medicinal plant, as well as applicable in developing new herbal products and functional foods in the future.

Phytochemistry

To date, a total of 70 compounds have been isolated and identified from leaves, fruits, barks and roots extracts of MPL, including 30 flavonoids, 11 alkaloids, 10 triterpenoids, 9 organic acids, 3 steroids and 7 other compounds. The Flavonoid were the most abundant components of MPL leaves and the content of flavonoids in leaves, stems and fruits of MPL were 16.94%, 5.15% and 1.52%, respectively. The Flavone in MPL leaves could be divided into flavones

including apigenin, 5, 6, 7, 8, 4′-pentamethoxyflavone and nobiletin, flavonols including isorhamnetin, kaempferol and quercetin, along with flavan-3-ols including catechin and epicatechin. Additionally, proanthocyanidin A2 was identified by liquid chromatograph-mass spectrometry (LC-MS). Flavonoids glycosides Flavonoid glycosides in MPL leaves could be classified as flavone O glycosides and flavone C-glycosides. The flavone-O-glycosides included nodifloretin-7-O-rhamnosyiglucoside,5,6,8,4′-tetrahydroxylflavone-7- O-rhamnoside, kaempferol-3-O-β-D-glucopyranoside, kaempferol-3-O-β-D-[3,6-di-p-(hydroxycinnamoyl)]-glucopyranoside, isorhamnetin-3-O-β-D- glucopyranoside, narcissin, kaempferol-3-O-β-D-(6-O-trans-p-coumaroyl)-glucopyranoside, typhaneoside, kaemferol-3-O-rutinoside and kaemferol-6-O-glucoside. Besides, kaemferol-7-O-glucoside, quercetin-3-O-rutinoside and apigenin-7-O-rutinoside were identified by high performance liquid chromatography (HPLC). The flavone C-glycosides included vitexin, isovitexin, violanthin, isoviolanthin, vicenin-1, vicenin-2, schaftoside and isoschaftoside. Alkaloids The alkaloids in MPL barks and leaves included N-methyl-6α-(deca-l′, 3′, 5′-trienyl)-3β-methoxy-2β-methylpiperidine isolated from the MPL stem barks as well as 6-(deca-l′, 3′, 5′-trienyl)-3-methoxy-2-methylpiperidine, N-methyl-6-(deca-l′,3′,5′-trienyl)-2,3-dimethylpiperidine, N-methyl-6-(deca-l′,3′,5′-trienyl)-2-methylpiperidine, microcosamine A, microcosamine B, microcosamine C, microgrewiapine A, microgrewiapine B, microgrewiapine C and erucicamide isolated from MPL leaves. Terpenoids The friedelin, arjunglucoside II were isolated from leaves chloroform extract; loliolide, (+)-dehydrovomifoliol were isolated from leaves ethyl acetate and n-butanol extracts; isololiolide, 3R, 6R-3-hydroxy-α-ionone, R-3-hydroxy-β-ionone were isolated from leaves ethyl acetate extract; 3β-O-p-hydroxy-E-cinnamoyloxy-2α, 23-dihydroxyolean-12-en-28-oate, 3-trans-feruloylmaslinic acid and maslinic acid were isolated from stems ethyl acetate extract. Organic acids The organic acids isolated from MPL leaves included isovanillic acid, p-coumaric acid, ferulicacid, abscisic acid, vanillic acid, syringic acid, methyl caffeate, p-hydroxybenzoic acid and chlorogenic acid. Steroids The stigmasterol, β-sitosterol and friedelinol were isolated and identified from MPL leaves. Others MPL leaves also contained saccharides such as sucrose and volatile oils whose main components were hydrocarbons and fatty acids. Besides, five lignan glycosides including (+)-lyoniresinol-2α-O-β-D-glucopyranoside, (+)- lyoniresinol-2α-O-α-L-rhamnopyranside, aviculin, (+)-syringaresinol-4′-O-β-D-glucoside, junipercomnoside and one megastigmadien glycoside called 3-hydroxy-7, 8-didehydro-β-ionol-9-O-β-D-glucopyranoside were identified from MPL leaves by LC-MS.[12-62]

PHARMACOLOGICAL ACTIVITIES

Antinociceptive Study

Formalin-Induced Paw Licking Test- The method of Hunskaar and Hole was used for the paw licking study. Thirty mice were divided into control group (distilled water), positive control or standard group (diclofenac sodium (DS), 100mg/kg bodyweight), and test groups (HMPB and PBMPB at 200 and 400mg/kg body weight), containing five mice in each group. The animals were fasted for 16h with water ad libitum. Mice in the control group, positive control group, and test groups received one dose of distilled water, diclofenac sodium, HMPB, and PBMPB orally. After 1h of treatment of each group, 2.7% formalin (v/v) at a dose of 20 μL was injected into the dorsal surface of the left hind paw of each mouse. The time spent for licking the injected paw was recorded. Animals were observed for 5min after formalin injection (acutephase) and for 5min in delayed phase, which was starting at the 20th minute after formalin injection.

The percentage of inhibition of licking was calculated using the following formula:
Inhibition (%) = [1 − Licking time (extract or standard drug) Licking Time (normal control)] × 100.[63-64]

Acetic Acid-Induced Writhing Test- The method of Koster et al. was employed for the writhing test. The animals were fasted for 16h with water ad libitum. Mice were pre-treated with extracts as mentioned before.DS (100mg/kg) was used as standard or positive control and distilled water as normal control. Forty-five minutes later, each mouse was injected intraperitoneally with 0.7% acetic acid at a dose of 10 mL/kg body weight. Fifteen minutes after the administration of acetic acid, the number of writhing responses was recorded for each animal during a 5-minute period. The percentage of inhibition of writhing was calculated using the following formula: Inhibition (%) = [1 − No. of writhing (extract or standard drug) No. of writhing (normal control)]× 100.[65]

Tail Immersion Test- The method of Toma et al. was employed for this test. The method was used to evaluate the central mechanism of analgesic activity. Here the painful reactions in animals were generated by thermal stimulus through dipping the tip of the tail in hot water. Mice were grouped and treated as described before. Tramadol (10mg/kg) was used as the reference drug. The animals were fasted for 16 h with water ad libitum. Before and after the treatment of each group, the basal reaction time, that is, time taken (in second) to withdraw it from hot water source, was measured by immersing the tail tips of the mice (last 1-2 cm) in

hot water of (55±1)∘C and the results were compared with control group. A latency period of 15 s was set as the cut-off point to avoid injury to mice. The latent period of the tail flick response was determined before 30min and after 30, 60, 120, and 180min of the respective treatment of each group.[66]

Hot Plate Test- Hot plate test was performed according to the method of Turner. The method was used to evaluate the central mechanism of analgesic activity. At first, mice were screened for this test by inserting them on a hot plate individually that was kept at (55 ± 1)∘C. The mice showing initial reaction time (difference of time between the placement of mice on hot plate and their responses to occur) of 15 s or less were selected for this study. A cutoff point of 15s was used to avoid the damage to the paw. Micewere grouped and treated as described before. Tramadol (10mg/kg) was used as the reference drug. The animals were fasted for 16 h with water ad libitum. 30min before the treatment of each group, the response latencies of mice were measured by placing them on hot plate after the observations of some parameters such as removal, jumping, or licking of the paws. The response latencies were also recorded after 30, 60, 120, and 180min of the respective treatment of each group.[67]

Gastrointestinal activity/ Digestion promoting activity

DAI et al. reported the digestion promoting activity of MPL leaves in rats for 7 days. The n-butanol, ethyl acetate and water extracts of MPL leaves (16.8 g·kg−1) showed a significant activity in promoting digestion, and their main mechanism was to diminish gastric juice pH and increase the amount of gastric juice and pepsin. With xiangsha yangwei pills (a TCM preparation for treating dyspepsia, 3 g·kg−1) as positive control, the water extract was conducted on intestinal ink propulsion test in mice. Though different groups (5.85, 11.7 and 23.4 g·kg−1) had no significant effect compared with the normal group, the high and medium dose groups showed an promoting trend in ink propulsion rate, suggesting that these groups may promote gastrointestinal motility mildly. However, the minimum effective dose of water extract (5.85 g·kg−1) used was too high, as compared with the traditional dosage of MPL leaves of 15−30 g·d−1 for human. Likewise, only single-dose (16.8 g·kg−1) was used for n-butanol and ethyl acetate extracts in that study.[68]

Antidiarrheal activity

Aziz et al. investigated the antidiarrheal activity of MPL fruits. The results indicated the chloroform extract of MPL fruits produced antidiarrheal activity in case of both of castor oil or MgSO4 induced diarrheal models. The inhibition of defecation at 200 and 400 mg·kg−1 (i.

g.) of MPL fruits reached to 77.78% and 83.33%, respectively for castor oil induced diarrheal mode in mice. While in MgSO4 induced diarrheal model, the inhibition of defecation at 200 and 400 mg·kg−1 (i.g.) was 80.65% and 90.32%, respectively.

In another study, Moushome et al. evaluated the antidiarrheal activity of hydromethanol and petroleum benzene extract of MPL barks. In case of castor oil induced diarrheal test, hydromethanol extract and petroleum benzene extract at 200 and 400 mg·kg−1 produced antidiarrheal activity in mice. The highest and significant percentage of inhibition of diarrhea (62.95%) was revealed by hydromethanol extract of MPL barks at 400 mg·kg−1. In case of MgSO4 induced diarrheal test, all of the extracts at doses of 200 and 400 mg·kg−1 also significantly decreased the total number of diarrheal feces. The Highest and most significant percentage of inhibition of diarrhea (68.13%) were revealed by petroleum benzene extract of MPL barks at 400 mg·kg−1. However, the highest does tested of MPL bark extracts was still less effective than the positive control loperamide HCL in both diarrheal models. Rahman et al. also found that methanol extract of MPL leaves (200 and 400 mg·kg−1, i.p.) could significantly alleviate diarrhea and enteropooling in mice. The extract also reduced gastrointestinal motility in charcoal meal test with the inhibition rate reaching to 20.34% at 200 mg·kg−1 and 29.49% at 400 mg·kg−1 (i.p.), respectively, while the inhibition rate of loperamide HCL (5 mg·kg−1, i.p.) was 40.52%. However, these extracts of MPL were extracted by organic solvents, which were different from the traditional decoction.[69-72]

Cytotoxicity Test

The brine shrimps used for cytotoxicity test were obtained by hatching 5 mg of eggs of in natural seawater after incubation at about 29°C for 48h. The larvae (nauplii) were allowed another 48 h in seawater to ensure survival and maturity before use. Six doses of plant extract (20, 40, 60, 80, 120 and 140 g/ml) in 5% DMSO and/or seawater were tested. Each extract preparation was dispensed into clean test tubes in 10 ml volumes and tested in duplicates. The concentration of DMSO in the vials was kept below 10l/ml. For control, same procedure was followed except test samples. After marking the test tubes properly, 10 living shrimps were added to each of the 20 vials with the help of a Pasteur pipette10. The test tube containing the sample and control were then incubated at 29°C for 24 h in a water bath, after which each tube was examined and the surviving nauplii counted. From this, the percentage of mortality was calculated at each concentration.[73-74]

MEMBRANE STABILIZING ACTIVITY TEST

Collection of Blood Samples 3 ml of blood was collected from each healthy Bangladeshi male human volunteer under standard condition. The collected blood was kept in a test tube with Ethylenediaminetetraacetic Acid (EDTA) to prevent clotting and stored until analysis. Erythrocyte Suspension The blood was rinsed by using isotonic solution (0.9% saline) for three times. The volume of saline was measured and reconstituted as a 40% (v/v) suspension with isotonic buffer solution (pH 7.4) which contained in 1 L of distilled water: NaH2PO4, 2H2O, 0.26 g; Na2HPO4, 1.15 g; NaCl, 9 g (10 mM sodium phosphate buffer). Thus, the suspension finally collected was the stock erythrocyte (RBC) suspension. Hypotonic Solution-Induced Hemolysis The membrane stabilizing activity of the extracts was evaluated by using hypotonic solution induced human erythrocyte hemolysis, which is established by Sikder with minor modification.[75] The experiments were carried out with freshly prepared hypotonic solution. The test sample consisted of stock erythrocyte (RBC) suspension (0.50 mL) with 5 ml of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffer saline (pH 7.4) containing either the different methanolic extract (2, 4, 6, 8, 10 mg/mL) or Acetyl Salicylic Acid (0.10 mg/mL). The Acetyl Salicylic Acid was used as a reference standard. The mixtures were incubated for 10 min at room temperature, centrifuged for 10 min at 3000 rpm and the absorbance of the supernatant was measured at 540 nm using Shimadzu UV spectrophotometer.[76]

The percentage inhibition of either hemolysis or membrane stabilization was calculated by using the following equation: % Inhibition of haemolysis = 100 × {(OD1- OD2)/ OD1} Where, OD1 = Optical density of hypotonicbuffered saline solution alone (control) and OD2 = Optical density of test sample in hypotonic solution. Thrombolytic Activity The thrombolytic activity of this plant methanolic extracts was evaluated by the method developed by Daginawala[77] and slightly modified by Ghosh et al.[78] where streptokinase was used as a standard. Commercially available lyophilized Altepase (Streptokinase) vial (Beacon pharmaceutical Ltd.) of 15, 00000 I.U. was collected and 5 ml sterile distilled water was added and mixed properly. 100µl (30,000 I.U) from this stock solution was used for in-vitro thrombolysis. Blood was drawn from five healthy human volunteers without a history of oral contraceptive or anticoagulant therapy. Then 1ml of blood was transferred to the previously dried and weighed micro centrifuge tubes and was allowed to form clots. Three replicates of each sample were prepared for statistical analysis and the values were represented as mean ± SD.[79] The thrombolytic activity of all extractives was evaluated by

the method developed by Daginawala volunteers and distributed in different pre weighed sterile micro centrifuge tube (1 ml/tube) and incubated at 37°C for 45 minutes. After clot formation, the clot is dried by removing serum completely and then each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube – weight of tube alone). To each micro centrifuge tube containing pre weighed clot, 100µl aqueous solution of different concentration of crude methanolic extract was added separately. Then, 100µl of streptokinase (SK) and 100 mg of distilled water were separately added with pre-formed clot as positive and negative controls, respectively. All the tubes were then incubated at 37°C for 90 minutes. After incubation, fluid generated over a certain period of time was removed and tubes were again weighed to observe the difference in weight after clot disruption.[80] Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis as shown below: % of clot lysis = (wt of released clot /clot wt) × 100

Antimicrobial and insecticidal activity

Aziz et al.[81] investigated the antibacterial activity of MPL fruits by employing agar disc diffusion test. With flucloxacilin (10 µg/disc) as positive control, the results implied that methanol extract exhibited antibacterial activity especially against gram negative bacteria, and the highest sensitivity was observed on P. mirabilis (zone of inhibition 28 mm). Likewise, both barks methanol extract and roots chloroform extract of MPL (2, 4 and 6 mg/disc) significantly slowed down the growth of gram-negative bacteria.[82]

Additionally, methanol and chloroform extracts from MPL barks, fruits and roots as well as and microcosamine A (35) and microcosamine B (36) exhibited the potential larvicidal activity against the 4th instar Culex quinquefasciatus mosquito. Similarly, N-methyl-6α-(deca-l′, 3′, 5′- trienyl)-3β- methoxy-2β-methylpiperidine suggested moribund toxic and growth-inhibitory bioactivities on the 2th instar larvae of the mosquito Aedes aegypti.[83] Both fruits chloroform and aqueous extracts of MPL (50 mg·mL−1) caused paralysis and death of Pheretima posthuma.[84,85] The antimicrobial and insecticidal activity of MPL barks, roots, fruits and leaves may partly explain the traditional detoxification bioactivity of MPL. However, the underlying mechanism and phytochemical analysis of MPL extracts is not proposed. Further studies on the structure activity relationship are necessary to ascertain its antimicrobial and insecticidal activity.

Anti-inflammatory and analgesic activity

Aziz et al. investigated the anti-inflammatory activity of MPL extracts by protease inhibition assay in vitro. The results showed that IC50 of fruits aqueous extract, fruits methanol extract and barks methanol extracts were 285.47, 201.55 and 61.31 μg·mL−1, respectively, while the IC50 of the Aspirin (positive control) was 24.46 μg·mL−1. In xylene-induced ear edema and cotton pellet-induced granuloma formation test examined by Aziz, methanolic extract of MPL barks and fruits (200 and 400 mg·kg−1) could suppress inflammation when compared with the diclofenac sodium treatment group (positive control), and fruits methanolic extract (400 mg·kg−1) implied the highest inhibition of 36.97% for ear edema and 45.96% for granuloma formation. At the molecular level, LI et al. evaluated the anti-inflammatory activity of apigenin C-glycosides from MPL leaves (10, 20 and 40 mg·kg−1) by lipopolysaccharide (LPS)-induced acute lung injury (ALI) model in mice. Apigenin C-glycosides attenuated ALI through modulating toll-like receptor 4 (TLR4)/ transient receptor potential channel 6 (TRPC6), reducing the release of pro-inflammatory cytokines and regulating the expression of apoptosis-related factors. Further study on structure-activity relationship of apigenin C-glycosides and ALI attenuating activity need to be carried out. The treatment for allergies of MPL barks, fruits and fruits may due to its inhibitory activity on inflammation. Oral treatment with methanol extract of MPL barks and fruits (200 and 400 mg·kg−1) significantly reduced paw licking of mice induced by formalin.[86]

The above extracts together with hydro-methanol and petroleum benzene extracts of MPL barks (200 and 400 mg·kg−1, i.g.) and ethanol extract of MPL leaves (250 and 500 mg·kg−1) were associated with the reduction of mice writhing induced by acetic acid. Moreover, in mice tail immersion test, barks petroleum benzene extract (200 and 400 mg·kg−1) suggested a significant increase in latency at 30 min, while barks methanol (400 mg·kg−1) and fruits methanol extract (200 and 400 mg·kg−1) suggested a significant increase in latency at 60 min compared with control group. In hot plate tests of mice, hydro methanol and petroleum benzene extracts (200 mg·kg−1) of MPL barks suggested a significant increase in response latency at 30 min compared with control group following the treatment.[87]

Anti-diabetic activity

Aziz et al. evaluated the in vitro α-amylase inhibitory activity of chloroform and aqueous extract of MPL fruits, and their IC50 were 1262.82 and 1367.56 μg·mL−1 compared to 785.84 μg·mL−1 of the positive drug acarbose. Likewise, CHEN et al.i nvestigated the α-

amylaes inhibitory activity of 70% methanol extract of MPL leaves along with vitexin, isovitexin and narcissin, and their IC50 were 61.30 μg·mL−1, 244.0, 266.2 and 275.4 μmol·L−1, respectively, while the IC50 of acarbose was 1007.0 μmol·L−1. However, the anti-diabetic activity of MPL only involved the exploration of α-amylase inhibitory activity in vitro, more in vivo studies are needed to reveal the anti-diabetic activity of compounds in MPL.[88]

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