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ANTIOXIDANT AND ANTIDIARRHEAL ACTIVITY OF THE METHANOLIC EXTRACT OF *MICROCOS PANICULATA* ROOTS

Aziz, M.A. [1]; Akter, M.I.[2]; Roy, D.N[1]; Mazumder, K[1]; Rana, M.S.[3]

[1]Department of Pharmacy, Jessore University of Science & Technology, Jessore-7408, Bangladesh

[2]Department of Pharmacy, Stamford University Bangladesh, 51, Siddeswari Road, Dhaka-1217, Bangladesh

[3]Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh

*debusubju@gmail.com

Abstract

Microcos paniculata is traditionally used for treating diarrhea, hepatitis, wounds, colds, fever, dyspepsia and heat stroke. *Objective*. To investigate the antioxidant and antidiarrheal activity of the methanolic extract of *M. paniculata* roots (RME). Antioxidant and antidiarrheal activity of RME were evaluated by different tests such as total antioxidant capacity (TAC), DPPH free radical scavenging assay (DPPHFRSA), nitric oxide scavenging capacity assay (NOSCA), lipid peroxidation by thiobarbituric acid assay (LPTAA), reducing capacity assessment (RCA), cupric reducing antioxidant capacity (CRAC), castor oil and $MgSO_4$ induced diarrheal tests. The total phenols and flavonoids contents and TAC were 182.78 ± 0.12 mg/g RME (in gallic acid equivalent) and 43.5 ± 0.32 mg/g RME (in quercetin equivalent) and 40.83 ± 0.69 mg/g RME (in ascorbic acid equivalent) respectively. The IC_{50} values of the RME in DPPHFRSA, NOSCA and LPTAA were 158.47 ± 2.66 µg/mL, 157.91 ± 4.56 µg/mL and 148.29 ± 6.48 µg/mL correspondingly. Concentration dependent reducing power was observed in RCA and CRAC respectively. Moreover, RME 400 mg/kg produced 68.10±16.99 % and 55.83±21.95 % inhibition of diarrhea in antidiarrheal models. Study results indicate that *M. paniculata* may provide a source of plant compounds with antioxidant and antidiarrheal activity.

Keywords: *Antioxidant, antidiarrheal, Microcos paniculata.*

Introduction

Free radicals are formed within tissues and membranes that initiate oxidative chain reactions which are impeded by antioxidants [1]. These free radicals are responsible for generating several diseases and can damage not only the DNA but also the cellular lipids and proteins. As a result, typical functions of humans are interrupted. However, antioxidants are useful against diabetes, aging, cancer, cirrhosis and atherosclerosis by inhibiting oxidative damages [2]. A range of synthetic antioxidants as like as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) etc. can cause toxicity as well as carcinogenicity to human and direct to the development of natural antioxidants [3-4].

Diarrhea can cause malnutrition, mortality and morbidity among children below 5 years of age. Enterotoxins, being the key reason of diarrhea in developing countries are formed and released by some microorganisms such as *Vibrio cholera, Salmonella, Shigella* and *Escherichia coli* [5]. Plants act as a vital source of antidiarrheal agents [6].

Microcos paniculata belongs to the family Tiliaceae having the local name as not only Kathgua but also Fattashi, which grows as like as shrub or small tree. It can grow naturally and distributed also in Bangladesh. By tradition, hepatitis, diarrhea, heat stroke, fever, dyspepsia, wounds and colds are treated by this plant. However, it is active against the digestive system, along with its insectisidal property. Several studies were performed for knowing some activities of this plant such as, antipyretic, larvicidal, cytotoxic, insecticidal, neuropharmacological, free radical scavenging, antidiarrheal, analgesic, anthelmintic, cytotoxic, α-glucosidase inhibition and antimicrobial. Besides, reports were found having its anti-inflammatory, brine shrimp lethality as well as nicotinic receptor antagonistic properties. In addition to, *M. paniculata* can also prevent both the angina pectoris and coronary heart disease. Additional reports revealed about its acute toxicity study [7-14].

Therefore, the present study was designed to evaluate the antioxidant and antidiarrheal activity of the methanolic extract of *M. paniculata* roots (RME).

Materials and Methods

Chemicals and Reagents

Ammonium molybdate, Folin-ciocalteu reagent, sodium nitroprusside, trichloroacetic acid (TCA), thiobarbituric acid (TBA), butylated hydroxytoluene (BHT), cupric chloride ($CuCl_2.2H_2O$), neocaproin, ammonium acetate buffer (pH 7), 1,1-diphenyl- 2-picryl-hydrazyl (DPPH), ascorbic acid, quercetin, and potassium ferricyanide [$K_3Fe(CN)_6$] were purchased from Merck, Germany. Loperamide HCl was purchased from the Bangladeshi manufacturer Square Pharmaceuticals Ltd. All other chemicals and reagents were of analytical grade and obtained from Sigma Chemical Co. Ltd., USA.

Plant Material

M. paniculata roots were collected from the Jahangirnagar University campus (23.8791° N, 90.2690° E), Savar, Dhaka, Bangladesh in November, 2013. Species identification was verified by Sarder Nasir Uddin, Principal Scientific Officer at the Bangladesh National Herbarium (accession number 35348). A dried specimen was deposited in the herbarium for future reference.

Preparation and Extraction of Plant Material

Methanolic extraction was carried out by using 200 g powdered roots of *M. paniculata*. Fresh roots were rinsed 3–4 times successively with running water and once with sterile distilled water. Washed plant material was then dried in the shade for a period of 7 d. The dried plant material was then ground by using a laboratory grinding mill (MACSALAB 200 Cross Beater, Eriez, Erie, Pennsylvania, U.S.A.) and passed through a 40-mesh sieve to get fine powder. Powdered fruits (200 g) was extracted in 2 L of methanol, using a soxhlet apparatus and a hot extraction procedure. Whatman No.1 filter papers were used to filter

the liquid extract. The filtrate was then dried in a hot air oven (BST/HAO-1127, Bionics Scientific Technologies Pvt. Ltd., Delhi, India) at 40 ℃. The extraction yield of RME was 2.84 % (w/w), which was stored at 4 ℃ for additional studies.

Experimental Animals

Thirty *Swiss albino* mice of either sex, 6–7 weeks old, weighting 25–30 g and five Sprague Dawley rats of either sex, 6–7 weeks old, weighting 150-190 g were collected from the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh, which were used in these experiments. These animals were kept under standard environmental conditions, having relative humidity 55 %–65 %, 12 h light/12 h dark cycle and (27.0±1.0) ℃ temperature. Proper supply of foods and water *ad libitum* were ensured. Before the experiment, animals were adapted to the laboratory conditions for 1 week. The Institutional Animal Ethical Committee of Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh approved the protocol used in these experiments conducted with these animals.

Acute Toxicity Study

Acute toxicity means when the adverse effects occur either from a single exposure or from multiple exposures within 24 h. Acute toxicity study can be conducted according to the direction of the OECD (Organization of Economic Cooperation and Development) guidelines and is required for knowing the LD_{50} of the plant extract [12, 15]. Both the control group and test group (RME) contained five rats. The rats of control group received normal saline at a dose of 10 mL/kg body weight orally, whereas the rats of test group received RME at various concentrations as like as 100, 250, 500, 1000, 2000, 3000 and 4000 mg/kg body weight through oral route. Then, these rats were monitored for collecting the data of mortality or several toxicities as like as weakness, diarrhea, injury, aggressiveness, noisy breathing, pain, convulsion, food or water refusal, salivation, changes in locomotor activity, discharge from eyes and ears, coma etc. At last, this acute toxicity study was assessed after monitoring these rats for two weeks [12, 15].

Determination of Total Phenolic Content

The total phenolic content of RME was determined by using Folin-Ciocalteu reagent [16]. 1 mL of RME or standard (gallic acid) of different concentrations (200, 100, 50, 25, 12.5 and 6.25 µg/mL) were taken in test tubes. 5 mL Folin-Ciocalteu (Diluted to 10 fold) reagent was added individually to these test tubes. Then, 7.5% (w/v) sodium carbonate solution (4 mL) was added separately into these test tubes and mixed well. Test tubes containing standard solutions were incubated for 30 min at 20℃ to complete the reaction but the test tubes containing RME solutions were incubated for 1 h at 20℃ to complete the reaction. Then the absorbance of these solutions was measured individually at 765 nm using a spectrophotometer (Shimadzu UV-1601 PC, Shimadzu Corporation, Japan) against blank. A typical blank solution contained the solvent used to dissolve the RME or standard. The total content of phenolic compounds in RME was expressed in mg/g gallic acid equivalent (GAE) and was calculated by the following formula.

C = (c x V)/m; Where, C = total content of phenolic compounds, mg/g RME in GAE; c = the concentration of gallic acid established from the calibration curve in mg/mL;V = the volume of RME in mL; m = the weight of RME in g.

Determination of Total Flavonoids Content

The total flavonoids content of RME was determined by using the aluminum chloride colorimetric method [17]. 1 mL of RME or standard (quercetin) of different concentrations (200, 100, 50, 25, 12.5 and 6.25 µg/mL) were taken in test tubes. After that, 3 mL of methanol was added individually to these test tubes. Then 200 µL of 10 % (w/v) aluminium chloride solution was added separately into these test tubes, followed by the addition of 200 µL of 1 M potassium acetate

solution into these test tubes.Finally, 5.6 mL distilled water was separately mixed with the reaction mixture of every test tube.Test tubes containing the reaction mixture were then incubated for 30 min at room temperature to complete the reaction.Then the absorbance of these solutions was measured individually at 415 nm using a spectrophotometer (Shimadzu UV-1601 PC, Shimadzu Corporation, Japan) against blank.The total content of flavonoid compounds in RME was expressed in mg/g quercetin equivalent (QE) and was calculated by the following formula.

C = (c x V)/m; where, C = total content of flavonoid compounds, mg/g RME, in quercetin equivalent; c = the concentration of quercetin established from the calibration curve in mg/mL; V = the volume of RME in mL; m = the weight of RME in g.

Evaluation of Antioxidant Activity

Determination of Total Antioxidant Capacity

Phosphomolybdenum method was employed for determining the antioxidant activity of RME [18]. 0.3 mL of RME or standard (ascorbic acid) of different concentrations (200, 100, 50, 25 and 5 µg/mL) were combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The test tubes, which contained reaction solutions were incubated at 95 ° C for 90 min to complete the reaction. Then the absorbance of these solutions was measured individually at 695 nm using a spectrophotometer (Shimadzu UV-1601 PC, Shimadzu Corporation, Japan) against blank after cooling at room temperature. A typical blank solution (without sample) contained the mixture of 3 mL reagent solution and the appropriate volume (300 µL) of the same solvent (ethanol) that was used for dissolving the sample and incubated under the same conditions as the rest of the sample solution. The antioxidant activity of RME was expressed as the mg/g RME in ascorbic acid equivalent (AAE) by the following formula.

C= (c x V)/m; Where, C = total antioxidant capacity, mg/g RME, in AAE; c = the concentration of ascorbic acid established from the calibration curve in mg/mL; V = the volume of RME in mL; m = the weight of RME in g.

DPPH Free Radical Scavenging Assay [19]

1 mL of RME or standard (ascorbic acid) of different concentrations (800, 400, 200, 100, 50, 25, 12.5 and 6.25 µg/mL) were taken in test tubes and were marked properly. Then 2 mL of 0.004% (w/v) DPPH solution was added to each test tube to make the final volume 3 mL. After that, the mixture of these test tubes were incubated in a dark place at room temperature for 30 min. Then the absorbance of these solutions was measured individually at 517 nm using a spectrophotometer (Shimadzu UV-1601 PC, Shimadzu Corporation, Japan) against blank. The ability to scavenge the DPPH free radical was calculated using the following equation.

% Inhibition =

$$(1 - \frac{\text{Absorbance of RME or standard}}{\text{Absorbance of blank}}) \times 100$$

IC_{50} is the concentration at which 50 % of the total DPPH free radical is scavenged/ neutralized and was determined by linear regression method from plotting % inhibition of DPPH free radical by RME and standard against their corresponding concentration in µg/mL.

Nitric Oxide (NO) Scavenging Capacity Assay [20]

4 mL of RME and standard (ascorbic acid) of different concentrations (200, 100, 50, 25 and 5µg/mL) solutions were taken in different test tubes and 1 mL of sodium nitroprusside (5 mM) solution was added individually to these test tubes. The test tubes were incubated for 2 h at 30 ℃ to complete the reaction. Then, 2 mL solution was withdrawn from the mixture of every test tube and separately mixed with 1.2 mL of griess reagent (0.5 % , w/v) and the absorbance of these solutions was measured at 550 nm using a spectrophotometer (Shimadzu UV-1601 PC, Shimadzu Corporation,

Japan) against blank. The percentage (%) inhibition activity was calculated from the following equation.

% Inhibition =

$$(1 - \frac{\text{Absorbance of RME or standard}}{\text{Absorbance of blank}}) \times 100$$

IC_{50} values of RME and standard were determined by linear regression method from plotting % inhibition of NO by RME and standard against their corresponding concentration in µg/mL.

Lipid Peroxidation by Thiobarbituric Acid Assay [21]

1mL of different concentrations (200, 100, 50, 25, 12.5 and 6.25 µg/mL) of RME or standard (BHT) was added to prelabeled test tubes. 1 mL of 0.15 M KCl was added to each test tube and 0.5 mL of Sprague Dawley rat liver homogenate was added to it later and mixed well. After that, 100 µL of 0.2 mM $FeCl_3$ solution was added to initiate peroxidation. The total mixture was incubated at 37 ºC for 30 min. After the incubation, the reaction was terminated with the addition of 2 mL of ice cold 0.25 N HCl containing 15 % (w/v) TCA, 0.38 % (w/v) TBA and 0.5 % (w/v) BHT. The reaction mixture was heated at 80 ºC for 60 min. These samples were cooled and centrifuged at 3000 rpm for 10 min., and the absorbance of these supernatants was measured at 532 nm using a spectrophotometer (Shimadzu UV-1601 PC, Shimadzu Corporation, Japan) against blank.

The percentage (%) inhibition of lipid peroxidation was calculated from the following equation.

% Inhibition =

$$(1 - \frac{\text{Absorbance of RME or standard}}{\text{Absorbance of blank}}) \times 100$$

IC_{50} values of RME and standard were determined by linear regression method from plotting % inhibition of lipid peroxidation by RME and standard against their corresponding concentration in µg/mL.

Reducing Capacity Assessment [22]

2.0 mL of RME or standard (ascorbic acid) of different concentrations (200, 100, 50, 25 and 5 µg/mL) were taken in different test tubes and 2.5 mL of 1 % (w/v) potassium ferricyanide $[K_3Fe(CN)_6]$ solution was added into each of the test tubes.Then, the test tubes were incubated for 10 min at 50°C to complete the reaction and 2.5 ml of 10 % (w/v) TCA solution was added into each of the test tubes.The total mixtures were centrifuged at 3000 rpm for 10 min. After that, 2.5 mL supernatant solution was withdrawn from each of the mixtures and mixed with 2.5 mL distilled water. 0.5 mL of 0.1% (w/v) ferric chloride ($FeCl_3$) solution was added to each of the test tubes. Then the absorbance of these solutions was measured at 700 nm using a spectrophotometer (Shimadzu UV-1601 PC, Shimadzu Corporation, Japan) against blank. However, if the absorbance of the reaction mixture increases, then the reducing power of it increases also [23].

Cupric Reducing Antioxidant Capacity [24]

500 µL of RME or standard (ascorbic acid) of different concentrations (200, 100, 50, 25 and 5 µg/mL) were taken in different test tubes. 1 mL of 0.01 M $CuCl_2.2H_2O$ solution, 1 mL of ammonium acetate buffer (pH 7), 1 mL of 0.0075 M neocaproin solution and distilled water (600 µL) were added into the every test tube and the final volume of the mixture was adjusted to 4.1 mL. The total mixtures were incubated for 1 h at room temperature. Then the absorbance of these solutions was measured at 450 nm using a spectrophotometer (Shimadzu UV-1601 PC, Shimadzu Corporation, Japan) against blank. Moreover, reduction of Cu^{2+} ion into Cu^{+} takes place in presence of the polyphenolic antioxidant substance [24].

Evaluation of Antidiarrheal Activity

Castor Oil Induced Antidiarrheal Test

The model of Shoba and Thomas was followed for carrying out the test [25]. Preliminary screening of animals was done by administering 0.5 mL of castor oil orally and those animals started diarrhea were selected finally for the test. Twenty mice were divided into control group (distilled water), positive control or standard group (Loperamide HCl, 3mg/kg body weight), and test group (RME at

200 and 400 mg/kg body weight), containing five mice in each group. The animals were fasted for 16 h with water *ad libitum*. Mice in the control group, positive control group and test group received one dose of distilled water, loperamide HCl and FME orally. After 30 min of the above treatments, each animal received 0.5 mL of castor oil orally for initiating diarrhea. Blotting paper lined individual cage was used for placing every animal. These blotting papers were changed at every hour. The number of diarrheal feces was recorded for a period of 4 h and the percentage inhibition of diarrhea was calculated for every group of animals.

$MgSO_4$ Induced Antidiarrheal Test

A similar procedure as for castor oil induced diarrhea was maintained for magnesium sulphate induced diarrheal model. Preliminary screening of animals for diarrhea was done by administering magnesium sulphate at a dose of 2 g/kg orally. Then, the animals were fasted for 16 h with water *ad libitum*. Mice were grouped and treated as described before. 30 min later of pre-treatments, magnesium sulphate was administered orally at a dose of 2 g/kg to the animals. Here, the number of diarrheal feces was also recorded for a period of 4 h and the percentage inhibition of diarrhea was calculated for every group of animals. [26].

Statistical Analysis

All the results are expressed as mean ± standard error (S.E.). IC_{50} values were calculated by linear regression equations (Microsoft Excel 2007; Microsoft, Redmond, Washington, USA). All tests were analyzed statistically by one-way ANOVA followed by Dunnett's t test. $P<0.05$, *vs.* control was considered to be statistically significant. All data were analyzed using SPSS software (version 16; IBM Corporation, New York, USA).

Results

Acute Toxicity Study

No mortality or signs of toxicity or behavioral changes were observed during the 14 days observation period in rats receiving doses up to 4000 mg/kg of RME (test group). The control group showed the same result. This demonstrates that the test group did not experience acute oral toxicity at the doses tested.

Determination of Total Phenolic and Flavonoids Content

The total phenolic and flavonoids contents of RME were calculated by using the standard curve of gallic acid ($y = 0.009x + 0.058$; $R^2 = 0.999$) (figure 1) and quercetin ($y = 0.005x - 0.005$; $R^2 = 0.996$) (figure 2) correspondingly.The total phenols and flavonoids contents were 182.78 ± 0.12 *mg/g* RME (in GAE) and 43.5 ± 0.32 mg/g RME (in QE) respectively (table 1).

Evaluation of Antioxidant Activity

Determination of Total Antioxidant Capacity

Total antioxidant capacity of the RME was calculated by using the standard curve of ascorbic acid ($y = 0.006x + 0.101$; $R^2 = 0.991$) (figure 3). Total antioxidant capacity of the RME was found as 40.83 ± 0.69 mg/g RME (in AAE) (table 2).

DPPH (1, 1-diphenyl-2-picrylhydrazyl) Free Radical Scavenging Assay

The IC_{50} values of RME and ascorbic acid are 158.47 ± 2.66 µg/mL and 68.22 ± 0.31 µg/mL respectively (table 3). The percentage scavenging of DPPH free radical was found to rise with increasing the concentration of RME (figure 4).

Nitric Oxide Scavenging Capacity Assay

The IC_{50} values of RME and ascorbic acid are 157.91 ± 4.56 µg/mL and 44.60 ± 2.40 µg/mL respectively (table 4). The percentage inhibition of nitric oxide production was illustrated in figure 5, where it was observed that the scavenging of nitric oxide by the extract was also concentration dependent.

Lipid Peroxidation by Thiobarbituric Acid Assay

The IC_{50} values of RME and BHT are 148.29 ± 6.48 µg/mL and 25.05 ± 0.03 µg/mL respectively (table 5). The percentage inhibition of lipid peroxidation was illustrated in figure 6, where, it was observed that the

percentage inhibition of lipid peroxidation by the extract was also concentration dependent.

Reducing Capacity Assessment

Transformation of Fe^{3+} to Fe^{2+} was examined for determining the reducing power capacity of any plant extract [22, 23]. As the absorbance of both RME and ascorbic acid was increased with increasing their concentration, so the reducing capacity of them was also increased with increasing their concentration (figure 7).

Cupric Reducing Antioxidant Capacity

Concentration dependent reducing power (i.e., reduction of Cu^{2+} ion into Cu^{+}) was observed for both RME and ascorbic acid with the maximum absorbance of 0.49 and 1.51, respectively at the highest concentration tested (200 ug/mL) (figure 8).

Evaluation of Antidiarrheal Activity

Castor Oil Induced Diarrheal Test

In case of castor oil induced diarrheal test, loperamide HCl and RME 200 and 400 mg/kg produced antidiarrheal effect in mice. Among them, only the loperamide HCl at a dose of 3 mg/kg significantly decreased (*P<0.05, *vs.* control) the total no. of diarrheal feces and produced highest and significant (*P<0.05, *vs.* control) percentage inhibition of diarrhea (85.09 ± 1.58 %). Again, the RME at 400 mg/kg produced 68.10 ± 16.99 % inhibition of diarrhea (table 6).

$MgSO_4$ Induced Diarrheal Test

In case of $MgSO_4$ induced diarrheal test, loperamide HCl and RME 200 and 400 mg/kg produced antidiarrheal effect in mice. Among them, only the loperamide HCl at a dose of 3 mg/kg significantly decreased (*P<0.05, *vs.* control) the total no. of diarrheal feces and produced highest and significant (*P<0.05, *vs.* control) percentage inhibition of diarrhea (74.84 ± 4.08 %). Again, the RME at 400 mg/kg produced 55.83 ± 21.95 % inhibition of diarrhea (table 7).

Discussion

Till now a few toxicity studies of plant derived products have been carried out. So, the acute toxicity study of plant extract can give idea about the appropriate range of doses for successive usage as well as to determine the probable adverse effects of it. Again, this study helps for determining the therapeutic index of drugs and xenobiotics [12]. LD_{50} of the RME could not be obtained, as no mortality was observed up to the dose as high as 4000 mg/kg and the extract was found to be safe with a broad therapeutic range. Therefore, two comparatively high doses (200 and 400 mg/kg) for RME was used for in-vivo doses.

Having hydroxyl groups, phenols can scavenge the free radicals. And these hydroxyl groups are responsible for the antioxidant activity of the phenols [27]. Furthermore, antioxidant properties of plants may be due to their phenolic as well as polyphenolic compounds which work as hydrogen donors, singlet oxygen quenchers, metal ion chelator and/or reducing agents [23].

Being secondary metabolites, flavonoids are commonly spread in the plant kingdom. The numbers of flavonoids which have been found in plants are more than 6000 [28]. Flavonoids of plants show their antioxidant properties by various ways, which are chelation of metal ions, such as iron as well as copper, hindrance of enzymes liable for free radical production and scavenging of free radicals [29]. There is a difference between the antioxidant activity and the total antioxidant capacity. First one point out the antioxidant characteristic of only one antioxidant, while the total antioxidant capacity (TAC) signifies the total antioxidant characteristic of all antioxidants. So, there is a possibility of getting more information by TAC than specific antioxidant ingredients [30]. The total antioxidant capacity of RME was evaluated by the phosphomolybdenum method according to the procedure described by Prieto *et al.,* which was based on the reduction of Mo (VI) to Mo (V) by the RME and the subsequent formation of green phosphate / Mo (V) at acidic P^H. Moreover, it is a quantitative procedure due to the expression of the total antioxidant capacity as

the number of equivalents of ascorbic acid [18]. In DPPH radical scavenging assays, RME showed dose dependent scavenging of DPPH radicals in a way similar to that of the reference antioxidant ascorbic acid (figure 4). This assay is used for testing the free radical scavenging activity of compounds or plant extracts [31-34]. DPPH is a stable free radical and the basis of this assay is the decolourization of it in the presence of antioxidants [35]. Hence, RME may donate electron to DPPH for decolourization of it that can be quantitatively measured from the changes in absorbance [36]. Production of NO occurs through neurons, endothelial cells and macrophages for controlling a range of biochemical processes. Different disorders such as Alzheimer's, AIDS, arthritis and cancer have link with the cytotoxic effects of NO when it is generated and assembled in excess amount [37]. There is no direct interaction between bioorganic macromolecules like DNA or proteins with NO. Various intermediates such as N_3O_4, NO_2, N_2O_4 and some stable products like nitrite and nitrate are produced when very unstable NO reacts with the oxygen in presence of aerobic conditions [38-39]. Besides, peroxynitrite is produced also in presence of superoxide and NO. Genotoxic reactions are also found by these substances. N_2O_3 has the ability to deaminate adenine, guanine and cytosine. The enzymatic functions of some thiol rich DNA repair proteins such as DNA ligase, DNA alkyl transferase, and formamopyrimidine-DNA glycosalase are affected by NO leading to genetic disintegrity. NO and its derivatives favour carcinogenesis process through their genotoxic effect [40]. In addition to, ischemic renal injury may be happened by NO and superoxide anion [41-43]. In the laboratory, NO is produced from sodium nitroprusside [44]. In the present study, RME exhibited nitric oxide radical scavenging activity, which may compete with oxygen to react with NO for inhibiting the generation of various products of NO.

Free radicals from different sources such as iron containing compounds, organic hydro peroxides and redox cycling compounds are responsible for generating lipid peroxidation. Reaction between malondialdehyde (MDA, a secondary product of lipid peroxidation) and TBA produces a red chromogen that can be determined through spectrophotometer [45]. In this study, RME might be capable of preventing the formation of MDA in a dose dependent manner. There is a relationship between the reducing properties and reductones. Mechanism behind the antioxidant action of reductones is the breakage of free radical chain by providing a hydrogen atom [23]. Here, the probable mechanism of antioxidant action of RME may be due to its hydrogen atom donating capability. The basis of the cupric reducing antioxidant capacity is the increase in the absorbance of the reaction mixtures that point out the increase in the antioxidant activity. Reducing power of any sample can give idea about the antioxidant property of it. So, if any compound shows reducing power capacity, then it can act as antioxidant through the donation of electron to the oxidized substance [46]. Therefore, the donation of electron to the Cu^{2+} may be the antioxidant mechanism of RME in the cupric reducing antioxidant capacity assay.

In the traditional medicine system, *M. paniculata* is used in the management of diarrhea [7]. Our results showed that the RME did not significantly inhibited ($p > 0.05$, *vs.*control) castor oil-induced diarrhea in mice. Antidiarrheal property of drugs or extracts can be evaluated by using castor oil induced diarrheal model. Ricinoleic acid is the active component of castor oil which causes irritation and inflammation of the intestinal mucosa. Peristaltic activity of the small intestine is stimulated by this irritation that causes change in the electrolytic permeability of the intestinal mucosa. After these events, prostaglandins are released which stimulate the secretion and motility of gastrointestinal

tract that decreases the absorption of potassium and sodium ions [7]. In this study, RME was found to inhibit the severity of diarrhea induced by castor oil (table 6). It is possible that the RME was able to inhibit the electrolyte permeability of the intestine and prostaglandin release. After oral administration of magnesium sulphate, accumulation of fluid in the intestinal lumen is increased, along with enhancing the fluid flow from proximal to the distal intestine. It may also release NO and liberates cholecystokinin from duodenal mucosa. Because of this, secretion and motility of small intestine are increased causing the prevention of reabsorption of water and NaCl [7]. Here, the RME reduced the percentage inhibition (table 7) of diarrhea that was expected due to the increase in electrolyte and water reabsorption from the gastrointestinal tract.

Conclusion

From the existing study, it could be suggested that the methanolic extract of *Microcos paniculata* roots (RME) might possess antioxidant and antidiarrheal activity. Nevertheless, further quantitative chemical studies are now under way to isolate and determine the structure of the active constituents. Similarly, we are seeking out biological testing of the specific compounds thought to be responsible for antioxidant and antidiarrheal activity presented in the RME. Again, genotoxicity study of this plant should be carried out for safety evaluation, though in the present study the RME did not show any acute oral toxicity.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Table 1: Total phenolic and flavonoids contents of RME.

Sample	Total phenols mg/g RME (in GAE)	Total flavonoids mg/g RME (in QE)
RME	182.78± 0.12	43.5± 0.32

Values are expressed as mean ± S.E. of triplicate experiments.

Table 2: Total antioxidant capacity of RME.

Sample	Total antioxidant capacity mg/g RME (in AAE)
RME	40.83 ± 0.69

Value is expressed as mean ± S.E. of triplicate experiments.

Table 3: IC_{50} values of the sample/standard in DPPH free radical scavenging assay.

Sample/Standard	IC_{50} (µg/mL)
RME	158.47 ± 2.66
Ascorbic acid	68.22 ± 0.31

The IC_{50} values are expressed as mean ± S.E. of triplicate experiments.

Table 4: IC_{50} values of the sample/standard in nitric oxide scavenging capacity assay.

Sample/Standard	IC_{50} (µg/mL)
RME	157.91 ± 4.56
Ascorbic acid	44.60 ± 2.40

The IC_{50} values are expressed as mean ± S.E. of triplicate experiments.

Table 5: IC_{50} values of the sample/standard in lipid peroxidation assay.

Sample/Standard	IC_{50} (µg/mL)
RME	148.29 ± 6.48
BHT	25.05 ± 0.03

The IC_{50} values are expressed as mean ± S.E. of triplicate experiments.

Table 6: Effect of standard and RME in castor oil induced diarrheal test.

Group	Dose	No. of diarrheal feces	% inhibition of diarrhea
Control	10 mL/kg	9.20 ± 0.86	0.00 ± 0.00
Loperamide HCl	3 mg/kg	1.40 ± 0.24*	85.09 ± 1.58*
RME	200 mg/kg	7.00 ± 2.60	18.69 ± 32.06
RME	400 mg/kg	3.20 ± 2.03	68.10 ± 16.99

Values are presented as mean ± S.E. n = 5 mice in each group. *P<0.05, *vs.* control (Dunnett's t test).

Table 7: Effect of standard and RME in $MgSO_4$ induced diarrheal test.

Group	Dose	No of diarrheal feces	% inhibition of diarrhea
Control	10 mL/kg	8.00 ± 0.32	0.00 ± 0.00
Loperamide HCl	3 mg/kg	2.00 ± 0.32*	74.84 ± 4.08*
RME	200 mg/kg	4.60 ± 2.20	44.44 ± 27.09
RME	400 mg/kg	3.60 ± 1.75	55.83 ± 21.95

Values are presented as mean ± S.E. n = 5 mice in each group. *P<0.05, *vs.* control (Dunnett's t test).

Figure 1: Calibration curve of gallic acid.

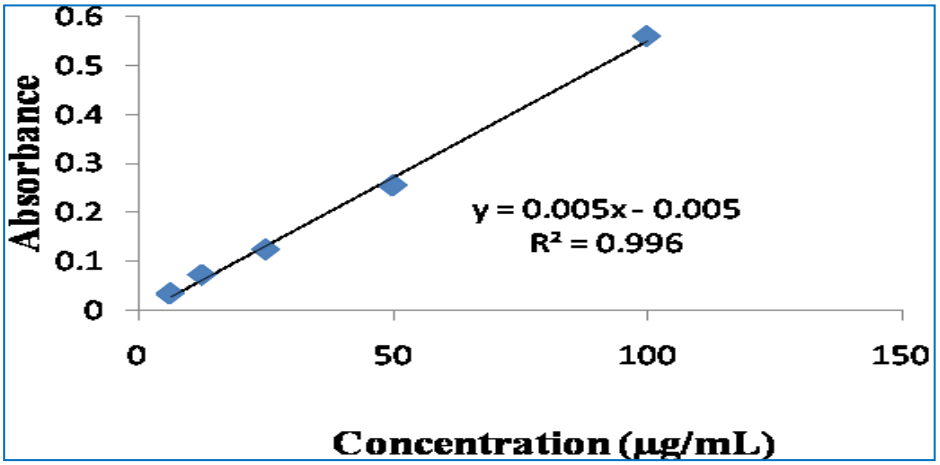

Figure 2: Calibration curve of quercetin.

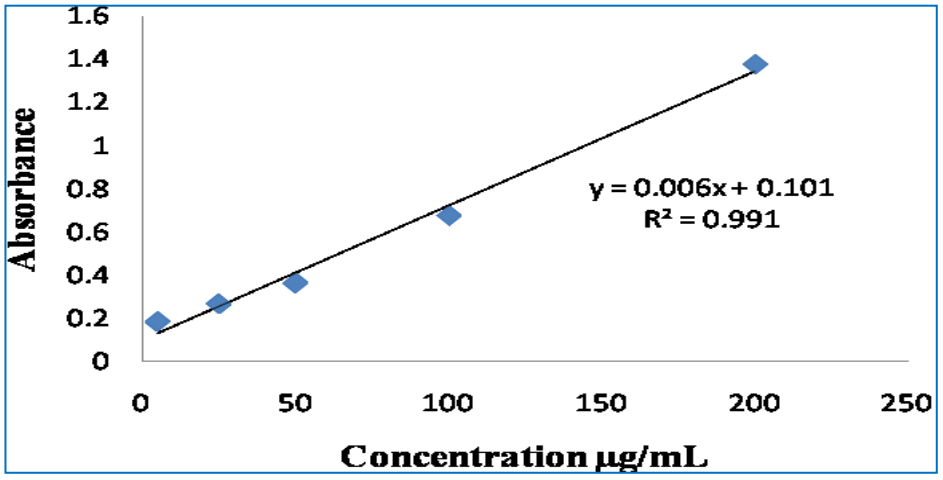

Figure 3: Calibration curve of ascorbic acid.

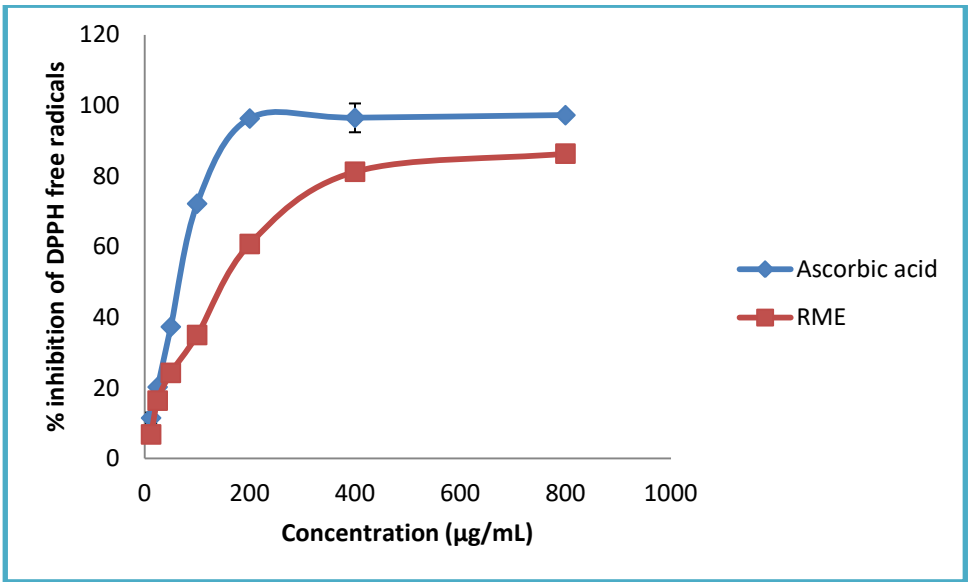

Figure 4: DPPH free radical scavenging activity of the RME and ascorbic acid. Values are presented as mean ± S.E. of triplicate experiments.

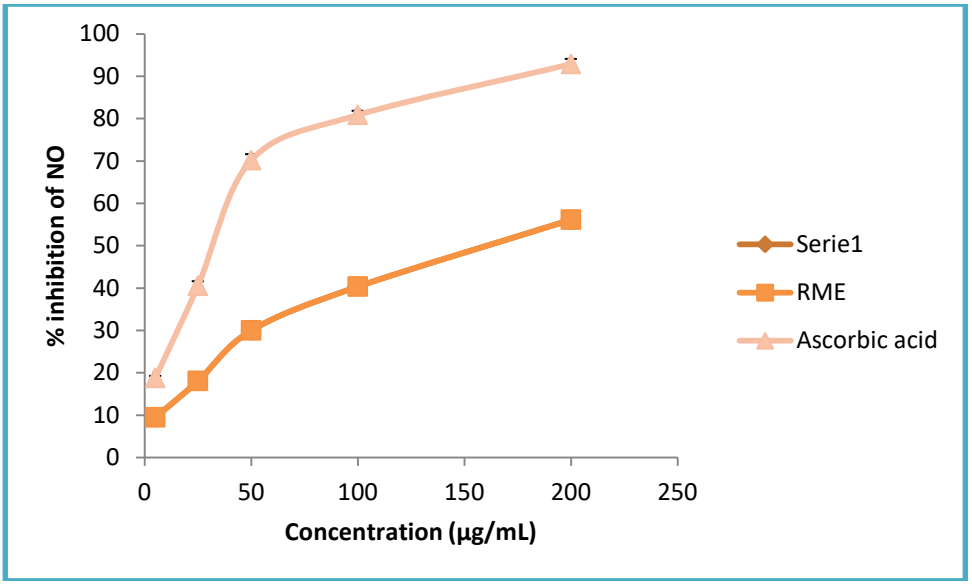

Figure 5: Nitric oxide radical scavenging activity of the RME and ascorbic acid. Values are presented as mean ± S.E. of triplicate experiments.

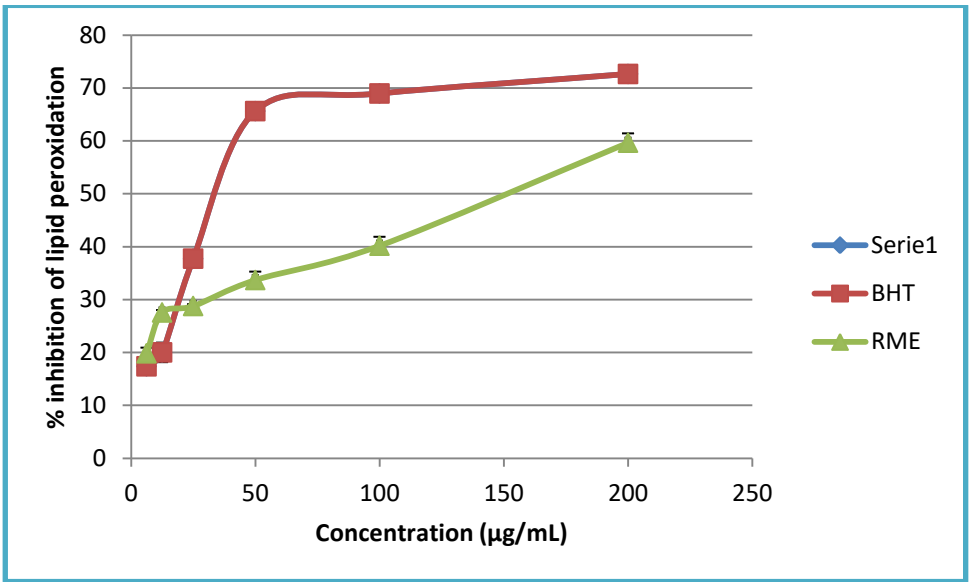

Figure 6: Lipid peroxidation inhibition activity of the RME and BHT. Values are presented as mean ± S.E. of triplicate experiments.

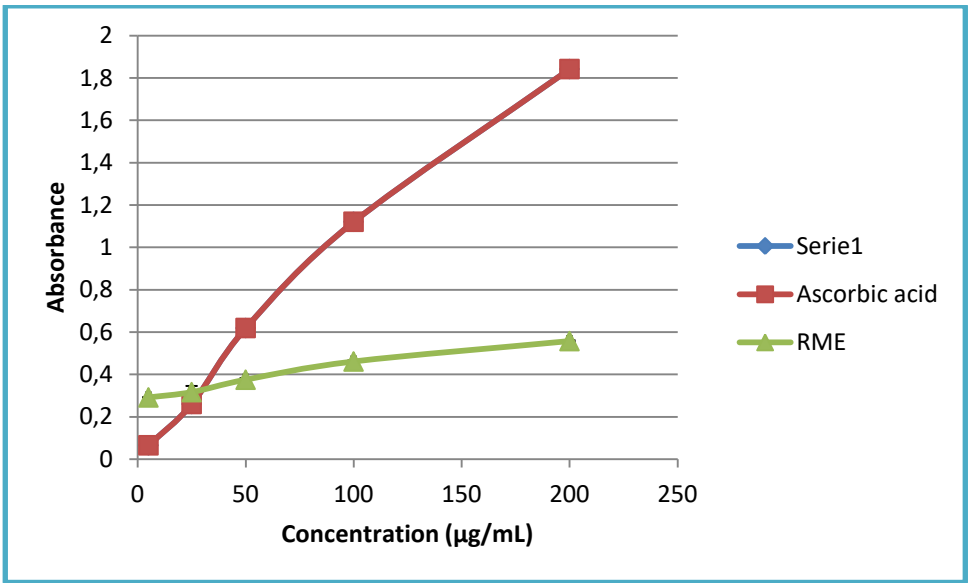

Figure 7: Reducing capacity of RME and ascorbic acid. Values are presented as mean ± S.E. of triplicate experiments.

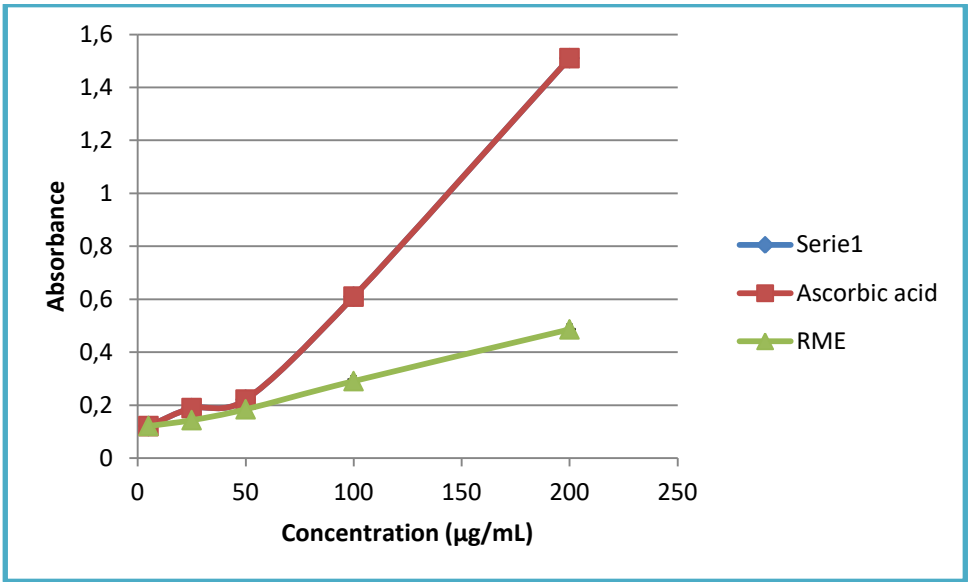

Figure 8: Cupric reducing capacity of RME and ascorbic acid. Values are presented as mean ± S.E. of triplicate experiments.