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Bark extract of *Chaetocarpus castanocarpus* (Roxb.) exhibits potent sedative, anxiolytic, and antidepressant effects through an *in vivo* approach in Swiss albino mice

R. RAHMAN[1], T. SIDDIQUE[1], F.A. NIPA[1], S. SULTANA[1], P. DEVI[1], F. ISLAM[2], F. NAINU[3], A.J. OBAIDULLAH[4], T.B. EMRAN[2,5,6], M.R. KHATUN[1]

[1]Department of Pharmacy, International Islamic University Chittagong, Chittagong, Bangladesh
[2]Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University, Dhaka, Bangladesh
[3]Department of Pharmacy, Faculty of Pharmacy, Hasanuddin University, Makassar, Indonesia
[4]Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia
[5]Department of Pathology and Laboratory Medicine, Warren Alpert Medical School, Brown University, Providence, RI, USA
[6]Legorreta Cancer Center, Brown University, Providence, RI, USA

Abstract. – OBJECTIVE: Standard phytochemical investigations were performed to identify the secondary metabolites in the methanol extract of *Chaetocarpus castanocarpus* bark (MECC) and investigate the neuropharmacological potential of MECC in Swiss albino mice.

MATERIALS AND METHODS: Swiss albino mice were used in the forced swimming test (FST) and tail suspension test (TST) to evaluate the antidepressant effect of MECC. Also, the hole board test (HBT) and elevated plus maze (EPM) were conducted to examine anxiolytic activities. In contrast, the open field test (OFT) and hole cross test (HCT) were employed to evaluate sleeping disorders.

RESULTS: Alkaloids, glycosides, flavonoids, terpenoids, coumarins, and tannins are only a few secondary metabolites identified in MECC by qualitative and quantitative phytochemical investigations. The oral administration of MECC considerably shortened the immobility duration during FST and TST. Encouraging dose-dependent anxiolytic effects were also observed in all relevant experiments compared to the control. Additionally, during the OFT and HCT assessment, a noteworthy decline in the locomotor activities of the experimental animals was observed.

CONCLUSIONS: The results of this investigation suggest that the *Chaetocarpus castanocarpus* bark is a possible source of therapeutic candidates for treating neurological disorders

Key Words:
Neuropharmacology, Sedative, Anxiolytic, Antidepressant, *In vivo*.

Abbreviations

5-Hydroxy Tryptamine (5-HT), Noradrenaline (NE), Dopamine (DA), Reactive Oxygen Species (ROS), Serotonin Reuptake Inhibitors (SSRIs), Serotonin-Noradrenaline Reuptake Inhibitors (SNRIs), Diazepam (DZM), Elevated plus maze (EPM) test, Hole-board test (HBT), Open field test (OFT), Hole cross test (HCT), Tail suspension test (TST), Forced swimming test (FST), Methanol Extract of Chaetocarpus castanocarpus bark (MECC).

Introduction

Neurological illnesses are increasing, significantly affecting people's personal and social lives[1]. Mental illness and behavioral disorders affect about 450 million people globally, making up 12.3% of the total disease burden. The three most common psychiatric problems are insomnia, depression, and anxiety, among a variety of behavioral disorders and mental illnesses[2].

A neurotic or psychotic state referred to as depression is typified by sadness, rejection, hopelessness, difficulty focusing, and alterations in sleep habits[3]. It is a chronic condition that alters a person's thoughts, feelings, and behavior[4]. The World Health Organization (WHO) estimates that 3.8% of people suffer from depression, with 5.7% of individuals over 60 years old and 5% of

Corresponding Authors: T.B. Emran, MD; e-mail: talhabmb@bgctub.ac.bd; M.R. Khatun, MD; e-mail: riniara@iiuc.ac.bd

adults (4% of men and 6% of women) falling into this category. According to a hypothesis based on the physiological underpinnings of depression, monoamine neurotransmitters like dopamine (DA), noradrenaline (NE), and 5-hydroxy tryptamine (5-HT) are absent in depression. However, research[5,6] has revealed that individuals with severe depression have higher levels of ROS in their blood and brains, indicating that oxidative stress may contribute to the development of depression. Anxiety is a mental health condition that is characterized by a variety of symptoms that are unrelated to medical conditions and psychological discomfort. It has also been connected to chronic diseases, low quality of life, and diminished performance[7]. According to reports[8], 20% of adults will eventually suffer from these ailments. According to the National Institute of Mental Health (NIMH) in the US, anxiety disorders and other mental/emotional illnesses, including depression and traumatic events, may be related. They may also be correlated to other medical conditions. Endocrine abnormalities, such as thyroid disorders, and issues with the body's control over glucose, such as diabetes and hypoglycemia, count as these conditions[8]. Similarly, insomnia is an additional psychological disorder identified by difficulties in initiating or maintaining sleep[9]. According to a survey[10], nearly thirty percent of adults report having symptoms of insomnia.

Several medications, such as tricyclic antidepressants (TCAs), monoamine oxidase inhibitors, selective serotonin reuptake inhibitors (SSRIs), serotonin-noradrenaline reuptake inhibitors (SNRIs), and atypical antidepressants, are prescribed for the management of depressive problems[11]. While treating anxiety disorders with medicine will not completely cure them, it may significantly reduce symptoms and lessen their frequency. Prescription medications that are frequently used to treat anxiety disorders include antidepressants, mainly those in the selective serotonin reuptake inhibitor (SSRI) class, and benzodiazepines, also referred to as "anxiolytics"[8]. The FDA has approved and prescribed the drugs Lorazepam, Clonazepam, and Alprazolam for the management of insomnia[12].

However, there are significant side effects related to CNS medications, including anticholinergic effects, abuse/dependence, amnesia, drowsiness, cognitive dysfunction, clinical effect delay, and sexual dysfunction[11]. All these symptoms have an impact on patient adherence to treatment. Tolerance is developed from long-term benzodiazepine use, and acute withdrawal symptoms might arise from stopping the medication[13]. However, long-term SSRIs use can have significant adverse effects[14]. Diazepam is an important medication for anxiety and sleep disorders, according to the WHO, and it has the most consistent history of potency and health. Despite its exceptional effectiveness, diazepam has several serious adverse effects, such as aggression, violent conduct, and disinhibition from paradoxical stimulation. Sometimes, excessive doses can cause hypotension and respiratory depression, especially when administered parenterally[1]. Thus, the quest for novel molecules with sedative, antidepressant, and anxiolytic qualities while posing minimal risk of side effects persists[15].

Human and animal health can be significantly enhanced, and natural products can prevent diseases. Natural medicinal compounds derived from plants are a promising alternative for treating neurological and mental illnesses. Many phytoconstituents found in medicinal herbs, fruits, or vegetables can slow down the progression of neurodegeneration and enhance memory and cognitive function[1]. Furthermore, over the last decade, there has been a notable advancement in the search for innovative pharmacotherapy derived from medicinal plants to treat psychiatric disorders[16]. Herbal products are being used more often in psychiatric practices as complementary or alternative therapies. Many herbal medications have also been studied[17] for their ability to treat various conditions in animal models.

Chaetocarpus castanocarpus (Roxb.) is a large evergreen tree with a maximum height of 45 meters. This plant also known as *C. pungens* and Muttul Jam (Locally); (Family: Peraceae) is native to Bangladesh, Myanmar, Indonesia, Cambodia, India, Sri Lanka, Thailand, Laos, and Vietnam[18]. It grows most commonly in Bangladesh's upland districts of Chittagong and Sylhet[19]. In the tribe's territory, this plant's leaf extract is traditionally used to alleviate inflamed joints[20]. In addition, the tender leaves are boiled and consumed raw with rice or diced and served as spinach[21].

This study used various test methods on mice to investigate the sedative, anxiolytic, and antidepressant effects of *Chaetocarpus castanocarpus* bark extract. We know no prior experimental research has been done to describe the antidepressant, anxiolytic, and sedative properties of *Chaetocarpus castanocarpus.*

Materials and Methods

Drugs and Chemicals

The source of the methanol was Sigma Chemical Company in St. Louis, MO, USA. Diazepam and fluoxetine hydrochloride, two common medications, were acquired from Square Pharmaceuticals Ltd. in Bangladesh. Tween 80 was purchased from BDH Chemicals in Leicestershire, United Kingdom, while normal saline (0.9% NaCl) was obtained from Social Marketing Company Ltd. in Bangladesh. Before being used, MECC was dissolved in saline containing 1% Tween 80, whereas the other medications were dissolved in an isotonic saline solution (NaCl 0.9%). The reagents and substances utilized in this investigation were all of analytical grade.

Extract Preparation

Collection of barks of *Chaetocarpus castanocarpus* was done from Hazarikhil Wildlife Sanctuary (22°37'11.94" N 91°41'44.78" E) Chattogram District, Bangladesh, in October 2022 and authenticated by the taxonomist Prof. Dr. Shaikh Bakhtear Uddin, Department of Botany, University of Chittagong, Bangladesh (Specimen No#201022-157). The unwanted adulterants were escaped by hand, and then the plants' materials were used in fresh water. Finally, cleaned plant materials were shade-dried at ambient temperature (25 ± 1°C) and powdered using a mechanical grinder to a mesh size of 1 mm (Sieve No. 10/44). The powder material (25 g) was soaked in methanol at 1:10 (plant sample: solvent = 25: 250 ml) at 25 ± 2°C for 120 h[22,23]. The supernatant was filtered by Whatman #1 filter paper (Whatman plc, Maidstone, UK) and then concentrated using a rotary evaporator (Buchi, R114, Switzerland) under reduced pressure to obtain 8.56 grams of crude methanol extract. The crude extract was stored at 4°C for subsequent investigation.

Experimental Animals

Male Swiss albino mice weighing 22-30 g at 6 weeks of age were purchased from the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR, B), an animal research branch. The animals were kept in typical laboratory circumstances, with a 12-hour natural day-night cycle, temperature of 23 ± 2°C, and relative humidity of 55-60%. They also had unlimited access to commercial pellet feed and water. Before the experiment began, the experimental animals were given 48 hours to become used to the holding area[24]. The International Islamic University Chittagong's Department of Pharmacy's Ethics Committee examined and approved the experimental procedure.

Study Plan

A set of experimental animals was divided into four groups, each consisting of three mice. These groups included a control group, a standard group, and two test groups. For the elevated plus maze (EPM), hole-board (HBT), open field (OFT), and hole cross (HCT) tests, standard medication diazepam (1 mg/kg, b.w., i.p.) was used; for the tail suspension test (TST) and forced swimming test (FST), standard drug fluoxetine HCl (20 mg/kg, b.w., p.o.) was employed. The control group received vehicle (1% Tween 80 in distilled water, 10 mL/kg, p.o.), while the test groups received oral MECC at dosages of 200 and 400 (mg/kg, b.w., p.o.), respectively. Before the tests, the reference medications (fluoxetine HCl and diazepam) were mostly given at 15 minutes, and MECC (200 and 400 mg/kg) or vehicle at 30 minutes.

Standard Phytochemical Analysis

Qualitative phytochemical analysis of MECC was evaluated by following the previously described method[25] to identify the presence of secondary metabolites, particularly flavonoids, alkaloids, quinones, glycosides, steroids, tannins, phenols, and terpenoids.

Antidepressant Activity

Forced swimming test (FST)

The antidepressant effect of MECC was assessed in Swiss albino mice using the FST method[26]. The experimental animals were kept apart in an open cylindrical container measuring 10 cm in height by 25 cm in diameter, with 19 cm of water at 25 ± 1°C. A 48MP smartphone camera (Redmi Note 10 Lite, model: 2109106A11., India) was used to record the test for six minutes, with the final four minutes being used to account for the entire immobility period and the initial two minutes being used for adaptation. All groups of mice(control, standard, and two test groups) received treatment following the study strategy. Mice were seen to be immobile when they floated still, except for movements required to maintain their head above the water. The test's reduced immobility duration was regarded as antidepressant-like activity.

Tail Suspension test (TST)

The TST represents the most straightforward and reliable way to assess antidepressant activity. The approach previously described was used to measure the overall duration of immobility caused by TST[27,28]. Mice were suspended 50 centimeters above the ground using cohesive tape, with each mouse positioned approximately 1 centimeter from the tip of its tail. A 48 MP smartphone camera (Redmi Note 10 Lite, model: 2109106A11., India) was used to record the test for six minutes. The final four minutes accounted for the immobility period, with the initial two minutes used for adaptation. All four groups of mice (control, standard, and two tests) received treatment following the study design.

Anxiolytic Activity

Elevated plus maze (EPM) test

Two open arms measuring 35 cm by 5 cm and two closed arms measuring 35 cm by 5 cm by 20 cm comprise the EPM. A 5 cm × 5 cm center square was used to link the arms. The device was elevated to a height of 25 cm above the ground, and two arms were united to form a central platform represented by the plus sign. After the dose was administered for thirty minutes, the experimental mice were positioned in the middle of the EPM apparatus, with their heads facing the open arms. Using a 48MP smartphone camera (Redmi Note 10 Lite, model: 2109106A11., India), the behavioral effects of the mouse were observed for five of the six minutes, with the first minute serving as an initial adjustment period for two different types of parameters (time spent in open arms and number of entries in the open arms)[24,29]. All four groups of mice (control, standard, and two tests) received treatment following the study design.

Hole board test (HBT)

The hole board instrument consisted of a wooden box (20 cm × 40 cm) with sixteen evenly spaced holes, raised to 15 cm above the ground. The bottom of the hole board was 25 cm above the ground, with a center-to-center distance of 10 cm between each hole. The experimental animals were put in the center of the box and allowed to wander around thirty minutes after the test doses were given[24,29]. Ultimately, a 48MP smartphone camera (Redmi Note 10 Lite, model: 2109106A11., India) was used to capture the mice's head dipping numbers through the holes for 5 of the 6 minutes, with the first minute being used for adaptation. The head dip was counted if both experimental animals' eyes disappeared into the hole.

Sedative Activity

Open field test (OFT)

The 60 cm × 60 cm × 60 cm white square box with 25 equal-sized (5 cm × 5 cm) squares comprised the plywood open field of the instrument. The instrument was used to observe Swiss albino mice's locomotive and emotional random behavior. The open field's area was split into two colored square blocks – white and black. The lighting conditions and the experiment were carried out in a quiet room. Mice were placed in the center of the floor, and a 48-MP smartphone camera (Redmi Note 10 Lite, model: 2109106A11., India) was used to count how many square blocks each mouse crossed. This information was then calculated for three minutes at intervals of zero, thirty, sixty, ninety, and one hundred twenty minutes[30,31].

Hole cross test (HCT)

The stainless-steel hole cross cage measured 30 cm by 20 cm by 14 cm in area. A 7.5 cm-tall divider with a 3 cm diameter opening was positioned in the center of the cage. The animals were positioned in the middle of the hole cross apparatus, and a 48MP smartphone camera (Redmi Note 10 Lite, model: 2109106A11., India) was used to record the number of holes crossed from one chamber to another. This data was then calculated over three minutes at intervals of zero, thirty, sixty, ninety, and one hundred twenty minutes[32].

Ethical Clearance

All animal experiments were conducted in the Department of Pharmacy, International Islamic University Chittagong, Bangladesh. The study protocol was approved by the Department of Pharmacy, International Islamic University Chittagong, Bangladesh (Ref: Pharm/P&D/200/16-22).

Statistical Analysis

GraphPad Prism Version 8.0 (GraphPad Software Inc., San Diego, CA, USA) was used to perform one-way analysis of variance (ANOVA) followed by Dunnett's test for antidepressant and anxiolytic activities and two-way ANOVA followed by Bonferroni post hoc tests for sedative activity. To explore the statistical differences for

Table I. The MECC was analyzed according to qualitative phytochemical profiling.

Phytochemicals	Name of the test	Appearance	Observation
Alkaloid	Wagner test	A reddish-brown color	++
	Mayer's test	Yellow color	++
Glycosides	Shinoda test	Deep brown ring	+
Cardiac Glycosides	Legal test	Brown color	+
Flavonoids	Lead acetate test	Fluorescence yellow	+
Phenols	$FeCl_3$ test	Violet color	-
Coumarins	Ammonia test	Green color	++
Tannin	Lead acetate test	Yellow color	+
Phlobatannins	HCl test	No reddish precipitation form	-
Xanthoproteins	Xanthoprotein test	No reddish-brown precipitation form	-
Cholesterols	General test	No red rose color	-
Triterpenoids	Salkowski's test	Reddish-brown color form	+
Resins	$FeCl_3$ test	No precipitation	-
Quinones	HCl test	Yellow color does not form	-

Sign (++) indicates the hugely present; (+) indicates the moderately present; and (–) indicates the absence of the phytochemical class.

this study, all test groups were compared with control groups using the gathered data, which were reported as Mean ± SEM. *p*-values were regarded as statistically significant if they were lower than 0.05.

Results

Qualitative Phytochemical Analysis

Using standard phytochemical procedures, a preliminary qualitative phytochemical screening was carried out to identify bioactive chemicals present in the crude methanol extract of *Chaetocarpus castanocarpus* (Roxb.) bark[33,34]. The qualitative phytochemical profiling of MECC identified alkaloids, glycosides, flavonoids, terpenoids, coumarins, and tannins (Table I).

Effects of MECC on Antidepressant Activity Test

Figure 1A-B shows the effects of MECC therapy on the length of immobility time for FST and TST. Comparing the oral administration of MECC (200 and 400 mg/kg, b.w.) and the posi-

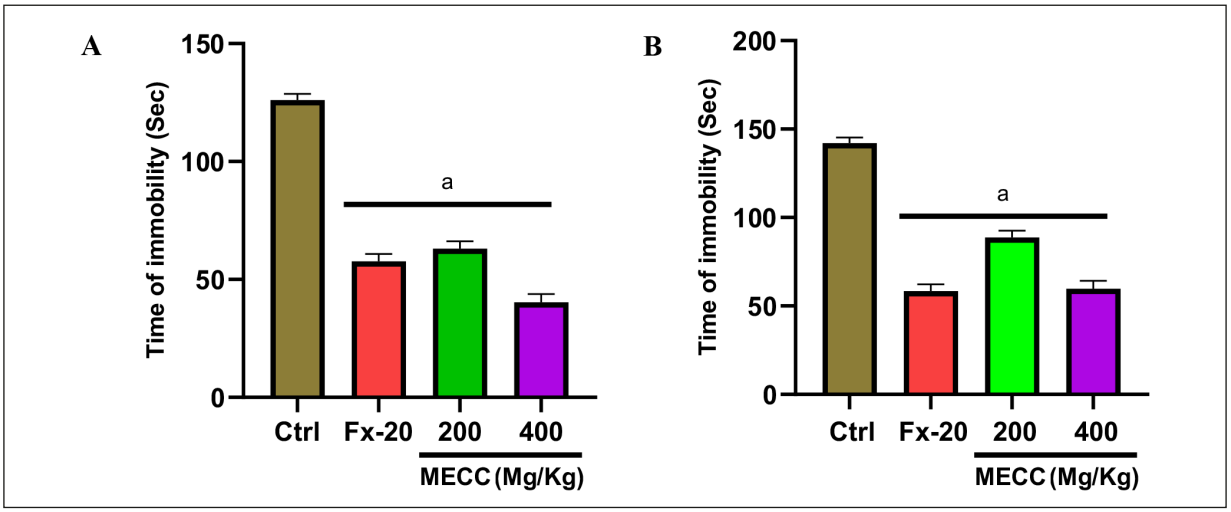

Figure 1. Effects of MECC on the immobility time of forced swimming test (**A**) and tail suspension test (**B**) in mice. The outcomes were displayed as mean ± SEM (n = 3), with statistically significant being defined as $^{d}p < 0.05$, $^{c}p < 0.01$, $^{b}p < 0.001$, and $^{a}p < 0.0001$ about the control group. Ctrl stands for control; DZM-1 for diazepam (1 mg/kg, i.p.); and MECC for *C. castanocarpus* bark methanol extract.

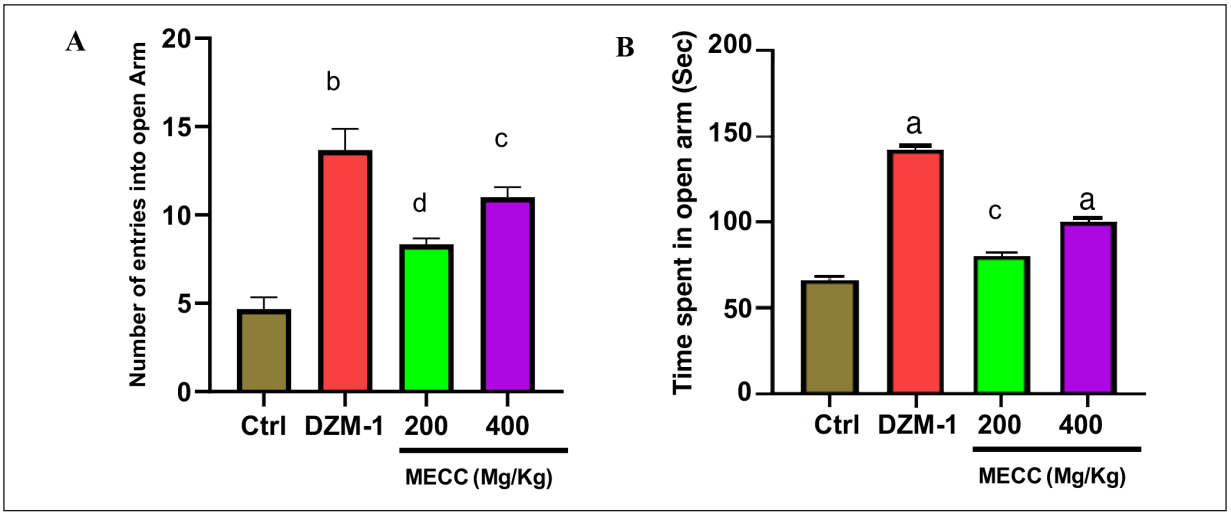

Figure 2. Effects of MECC on the number of entries into the open arm (**A**) and time spent in the open arm (**B**) in the EPM test. The outcomes were displayed as mean ± SEM (n = 3), with statistically significant being defined as $^{d}p < 0.05$, $^{c}p < 0.01$, $^{b}p < 0.001$, and $^{a}p < 0.0001$ concerning the control group. Ctrl stands for control; DZM-1 for diazepam (1 mg/kg, i.p.); and MECC for *C. castanocarpus* bark methanol extract.

tive control, fluoxetine HCl (20 mg/kg, b.w.), to the negative control, the figure demonstrated a significant [F(3, 8) =l40.6; $p < 0.0001$] reduction in the immobility duration in FST and a significant [F (3, 8) = 98.85; $p < 0.0001$] reduction in the immobility duration in TST.

Effects of MECC on Anxiolytic Activity Test

Elevated plus maze test

Following the results of the EPM test, MECC at two different dosages (200 and 400 mg/ kg) increased the number of entries and the duration of time spent in open arms in a dose-dependent way (Figure 2A-B). Figure 2A represents that [F (3, 8) = 25.32; $p < 0.0002$] the number of entry into the open arm increased significantly, and Figure 2B represents that the [F (3, 8) = 386.4; $p < 0.0001$] time spent in open arm increased significantly. Comparing the different doses to the control group, the doses of MECC at 400 mg/kg, b.w. and the positive control, diazepam (1 mg/kg, i.p.), had a highly significant effect.

Hole Board Test

The treatment exhibited modest activity when comparing the experimental animals treated with MECC at a higher dose to the control group in HBT. In contrast to the control group, the oral administration of MECC at a higher dose (400 mg/kg, b.w.) significantly [F (3, 8) = 83.68; $p < 0.0001$] increased the number of head dips (Figure 3). A similar outcome was observed when the usual medication diazepam (1 mg/kg, i.p.) was administered.

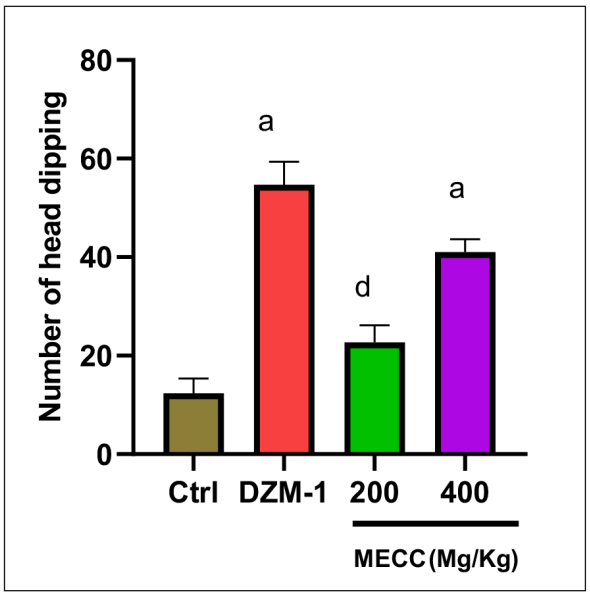

Figure 3. Effects of MECC on the hole board test in mice. The outcomes were displayed as mean ± SEM (n = 3), with statistically significant being defined as $^{d}p < 0.05$, $^{c}p < 0.01$, $^{b}p < 0.001$, and $^{a}p < 0.0001$ concerning the control group. Ctrl stands for control; DZM-1 for diazepam (1 mg/kg, i.p.); and MECC for *C. castanocarpus* bark methanol extract.

Effects of MECC on Sedative Activity Test

Open field test (OFT)

The reduction in square block crossing from the initial monitoring period (0 min) to the end of the monitoring period (120 min) in OFT indicated the experimental mice's locomotion activity. The results of two-way ANOVA showed significant [Interaction [F (12, 40) = 7.399; $p < 0.0001$], Row factor [F (4, 40) = 246.8; $p < 0.0001$], Column factor [F (3, 40) = 121.5; $p < 0.0001$]. When comparing MECC-400 with the control, the number of squares traversed was considerably ($p < 0.005$; $p < 0.001$ and $p < 0.0001$) reduced after oral administration of the drug at nearly all tested dosages. This indicates that inhibition started at 30 minutes and lasted until 120 minutes of closed monitoring. Meanwhile, the reference medication-treated group with diazepam (1 mg/kg, i.p.) exhibited comparable results (Figure 4A).

Hole cross test (HCT)

Figure 4B represents the MECC's record of spontaneous locomotor activity at various dosage levels. The results of two-way ANOVA showed significant [Interaction (F (12, 40) = 7.399; $p < 0.0001$), Row factor [F (4, 40) = 246.8; $p < 0.0001$], Column factor [F (3, 40) = 121.5; $p < 0.0001$].

Comparing the experimental mice to the control group, their overall mobility was considerably reduced ($p < 0.0001$) throughout the last three examination periods (60, 90, and 120 min) at all tested doses (200 and 400 mg/kg) of MECC, including the reference medication diazepam (1 mg/kg, i.p.) (Figure 4B).

Discussion

The physiological and therapeutic actions of the plant extract were also shown by phytochemi-

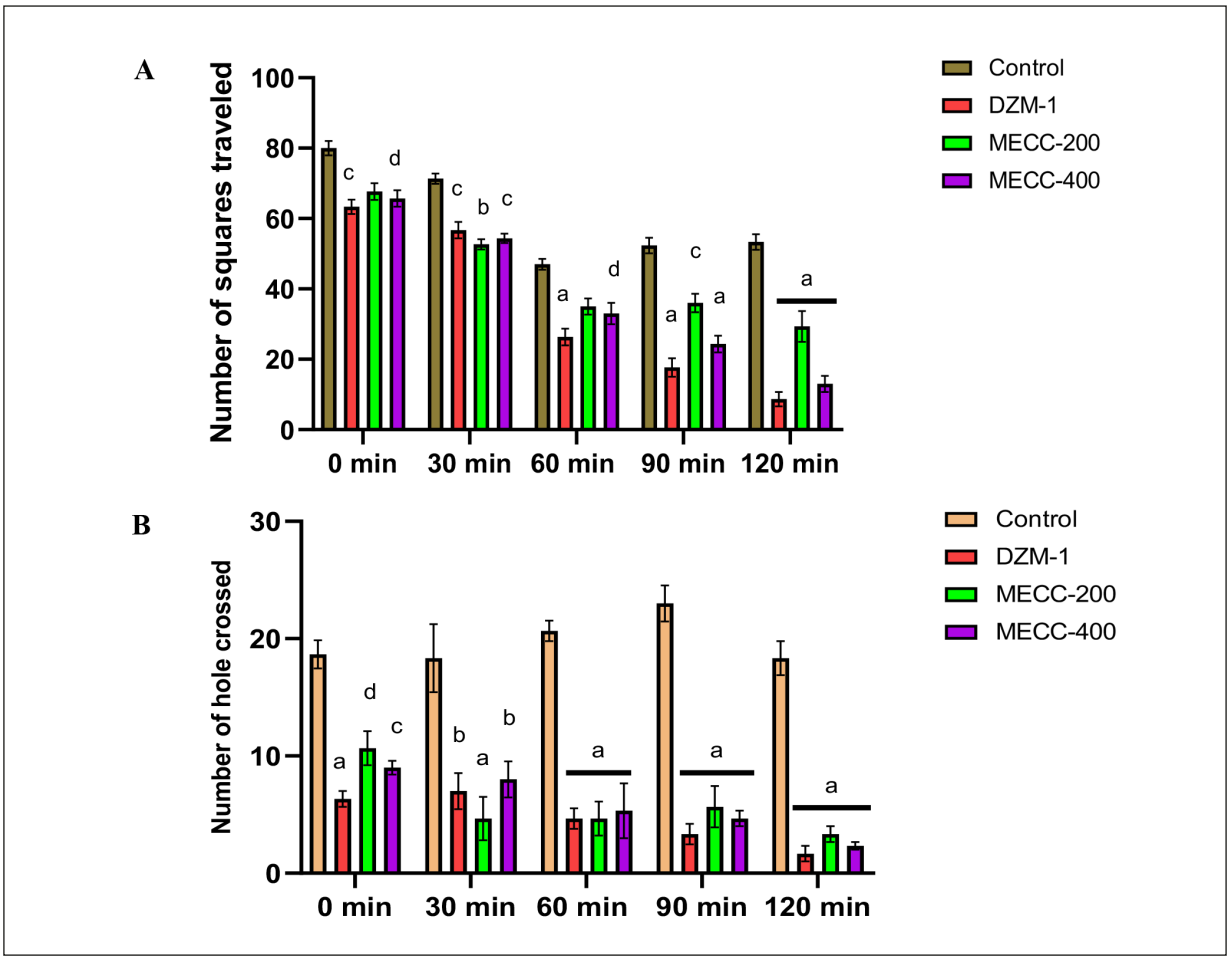

Figure 4. Effects of MECC on the open field test and hole cross test: the number of squares traveled (**A**) and the number of holes crossed (**B**) at the different intervals (0 min, 30 min, 60 min, 90 min, and 120 min). The outcomes were displayed as mean ± SEM (n = 3), with statistically significant being defined as $^{d}p < 0.05$, $^{c}p < 0.01$, $^{b}p < 0.001$, and $^{a}p < 0.0001$ concerning the control group. Ctrl stands for control; DZM-1 for diazepam (1 mg/kg, i.p.); and MECC for *C. castanocarpus* bark methanol extract.

cal analysis[35]. According to the phytochemical investigation, numerous secondary metabolites are present in *C. castanocarpus*. At the same time, alkaloids are a specific class of secondary nitrogenous chemicals that were once employed to cure a variety of middle-aged human and animal disorders[36]. Flavonoids are another important class of phytochemicals employed as anti-inflammatory, cancer, and cardiovascular treatments. Flavonoids are often ingested through food[37]. Natural remedies from traditional medicinal plants have been used for their curative properties since prehistoric times. Natural products are frequently used in the pharmaceutical, nutraceutical, and food supplement sectors to make various herbal medications, minerals, and dietary supplements[38]. To the best of our knowledge, no research has been published on the neuropharmacological effects of *C. castanocarpus* bark.

For this reason, the objective of the current study is to thoroughly investigate the potential implications of MECC on the central nervous system using an animal neurobehavioral model. Because of their bioactive secondary metabolites, medicinal plants and herbs have specific pharmacological properties necessary for their usage. The present study's qualitative phytochemical profile identified several primary phytoconstituents, including alkaloids, glycosides, flavonoids, terpenoids, coumarins, and tannins.

Depression and anxiety are related and typically coexist as states, and managing these two conditions improves the outcome of therapy[39]. In addition to being the first line of treatment for depression, serotonergic antidepressants, or SSRIs, have been shown to have significant anxiolytic effects[40]. Recently, the effects of a few kinds of drugs that affect serotonin (5-HT) neurotransmission have been studied in connection with schizophrenia and depression[41]. Several medicinal plants, including *M. angolensis*[42] and *L. angustifolia*[43], have been shown to exhibit both anxiolytic and antidepressant properties. The current experimental setup confirms that MECC has anxiolytic-like and antidepressant properties. The tail suspension test (TST) and forced swimming test (FST) assessed the depressive-like state in Swiss albino mice[44]. To illustrate the animal's behavioral desperation, similar to that observed in human depression, immobility or desperation behavior produced in both FST and TST has been rebuilt. The current experiment's results demonstrated MECC's considerable anxiolytic and antidepressant capabilities. FST and TST's immobility times were notably brief compared to control at lower and higher doses. According to statistical analysis, the immobility time for MECC (200 and 400 mg/kg) and standard medicine fluoxetine was significantly ($p < 0.0001$) reduced with both FST and TST compared to the control. A comparable immobility duration was seen in the case of fluoxetine HCl, a common antidepressant. Furthermore, research[45] on antidepressants has shown that normal antidepressant activity is regulated by reducing various stress parameters and monoaminergic levels and enhancing noradrenaline and serotonergic transmission in the brain, which supports the apoptogenic impact of the plant. Nevertheless, the precise process remains undetermined by our analysis; hence, further comprehensive mechanistic studies are required to elucidate the matter.

The elevated plus maze (EPM) or hole-board test (HBT) was often utilized for the preliminary anxiolytic activity study[46]. Anxiolytic effects were brought on by the brain's elevated GABAergic neurotransmission systems[47]. Typically, the EPM test is used for assessing anxiolytic substances in animals[48]. Time spent in the open arm exhibited activity in EPM in a dose-dependent way, while treatment with MECC (400 mg/kg) increased time in the open arm ($p < 0.0001$) when compared to the control. MECC-200 ($p < 0.05$) and MECC-400 ($p < 0.01$) (mg/kg) exhibited a statistically significant dose-dependent activity for open-arm entries, while the standard medication diazepam exposed ($p < 0.001$) open-arm entries. The HBT is a fundamental tool for assessing an animal's emotionality and anxiety reactions and how the animal would behave in a novel situation[49]. The increased number of heads dipping into the apparatus's hole signifies the antianxiety features[50]. Compared to 200 mg/kg, MECC at 400 mg/kg showed more head dipping. MECC-400 mg/kg and the common medication diazepam showed a significant outcome ($p < 0.0001$). According to the results above, the open arm time in EPM at 400 mg/kg of MECC exhibited a substantial ($p < 0.0001$) anxiolytic behavior. Additionally, another parameter (entries into the open arm) demonstrated a statistically insignificant activity ($p < 0.01$). HBT showed a similar outcome, but MECC showed anxiogenic-like effects at both doses. These results demonstrate the anxiogenic character of MECC, necessitating additional testing of these factors in the behavioral investigation.

The most common animal models, which include the open field test (OFT) and the hole cross test (HCT), were used to assess the sedative effects of MECC, identifying unrestrained locomotion activity. Relating to the benzodiazepine class, diazepam is a central nervous system depressant prescribed to treat insomnia along with other sleeping disorders. The GABA receptor complex contains a binding site for benzodiazepines. These medications reduce activity, control excitement, and relax the patient. Benzodiazepines also reduce sedative exploratory behavior and lengthen and delay the onset of barbiturate sleep[32]. The outcomes of the investigation demonstrated that in both tests, the sedative effect of MECC results in acceptable behavioral alterations. Notably, in both animal models, the degree of movements from one chamber to another and the number of hole crossings reduced significantly, indicating a decrease in the mice's locomotor activity. Compared to the control group, there was a significant, dose-dependent decrease in square movements when 200 and 400 mg/kg of MECC were given. Cross-hole and square motions were reduced between 30 and 120 minutes. The mice's altered behavior after being given MECC could indicate that medicinal compounds that improve motor function[51] and prevent the animals from moving around actively in OFT are present. Significant dose-dependent reduction in locomotion has been observed by MECC, which may have sedative and central nervous system (CNS) depressive effects *via* increasing hyperpolarization-induced γ-aminobutyric acid-ergic (GABAergic) inhibition. γ-aminobutyric acid (GABA) receptors may be activated, or neuronal activity may be reduced due to these effects[52]. However, as it is yet unclear whether specific components caused these effects, more research is required to assess the role of other chemicals that have been isolated for the reported activity.

Conclusions

The current study demonstrates the ethnomedical use of *Chaetocarpus castanocarpus* (Roxb.) bark for the treatment of neurodegenerative illnesses by revealing the plant's highly favorable neuropharmacological characteristics, which are attributed to the presence of many bioactive compounds. As a result, the current study suggests that the plant's bark could be a source of medication candidates for treating depression, sleeplessness, and other neurological conditions. However, a thorough mechanical analysis and subsequent bioactive lead isolation are essential to validate the precise mechanism underlying this unique plant's neuropharmacological insights.

Conflict of Interest
The authors declare that they have no conflict of interests.

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Ethics Approval
The study protocol was approved by the Department of Pharmacy, International Islamic University Chittagong, Bangladesh (Ref: Pharm/P&D/200/16-22).

Informed Consent
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Authors' Contribution
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ORCID ID
T.B. Emran: 0000-0003-3188-2272
M.R. Khatun: 0000-0001-9188-3977
F. Islam: 0000-0002-8167-8264
F. Nainu: 0000-0003-0989-4023
R. Rahman: 0009-0002-3161-3136
F. A. Nipa: 0009-0005-3025-2772
S. Sultana: 0009-0008-9941-6642
T. Siddique: 0009-0003-7612-7593
P. Devi: 0009-0001-9880-7851

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