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Anxiolytic, antidepressant and antioxidant activity of the methanol extract of *Canarium resiniferum* leaves

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ABSTRACT

Background and aim: This study evaluated the anxiolytic, antidepressant, and antioxidant activity of the methanol extract of *Canarium resiniferum* (MECR) leaves, and determined the total phenolic and flavonoid contents in this extract.

Experimental procedure: The anxiolytic effect of MECR (100, 200, 400 mg/kg, p. o.) was tested in mice using the elevated plus-maze (EPM) test, the hole-board test (HBT), and the light-dark box (LDB) test. Its antidepressant effect was evaluated in the tail suspension (TST) and the forced swim (FST) tests. The total phenolic (TPC) and flavonoid (TFC) content was measured using standard colorimetric assays. Antioxidant activity was determined using the DPPH radical scavenging and ferric reducing antioxidant power (FRAP) assays.

Results and conclusion: MECR, at all doses, showed dose-dependent anxiolytic activity. At 400 mg/kg, it significantly increased the time spent and number of entries in the open arms (EPM test), the number of head-dips (HBT), and the time spent into the light compartment (LDB) test compared to the control. In the TST and FST, MECR dose-dependently reduced the duration of immobility compared to untreated animals. This was significant for all doses except for 100 mg/kg in the FST model. MECR showed high TPC and TFC (90.94 ± 0.75 mg GAE/g and 51.54 ± 0.78 mg QE/g of dried extract, respectively) and displayed potent activity in the DPPH radical scavenging (IC_{50} = 177.82 μg/mL) and FRAP assays. These findings indicate that *C. resiniferum* has the potential to alleviate anxiety and depression disorders, which merits further exploration.

1. Novelty

Although the genus *Canarium* has been extensively previously studied for its biological activity, our findings are the first to report on the pharmacological activity of *C. resiniferum*. The potential to alleviate anxiety and depression disorders exhibited by this species

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List of abbreviations

MECR	Methanol extract of *Canarium resiniferum*
EPM	Elevated plus-maze test
HBT	hole-board test
LDB	Light-dark box test
TST	Tail suspension test
FST	Forced swim test
TPC	Total phenolic content
TFC	Total flavonoid content
FRAP	Ferric reducing antioxidant power assay
QE	Quercetin equivalents
GAE	Gallic acid equivalents

warrants further investigation as a safe alternative treatment for anxiety and depression.

2. Introduction

Canarium resiniferum Bruce ex King (Burseraceae) is a large evergreen tree native to Bangladesh and the Assam state of India.[1] In Bangladesh, the plant known as Dhup, is used by traditional medicinal healers for its resin which is commonly applied for the topical treatment of eczema.[2] Extracts or phytoconstituents of *Canarium* species have demonstrated a wide range of biological effects including hepatoprotective, analgesic, antimicrobial, anti-hypercholesterolemic, antioxidant, vasorelaxant, antiviral, anti-obesity, antidiabetic, antipyretic, anti-inflammatory, anticancer, α-amylase and α-glucosidase inhibitory activity.[3–13] To the best of our knowledge, *C. resiniferum* has yet to be explored for its phytoconstituents and pharmacological activity.

Anxiety disorders and depression are common mental disorders with symptoms that range from mild to severe. Anxiety disorders are characterized by a feeling of fear, often chronic, in response to the presence of threatening or unfamiliar situations. Depressive disorders are characterized by symptoms such as loss of interest, sadness, sleeplessness, poor appetite, the inability to perform daily tasks, and in severe cases a tendency to commit suicide.[14] Many of the current anxiolytic and antidepressant drugs exhibit undesirable side effects that contribute to poor patient compliance with the treatments.[15,16] This has been associated with an increase in the demand for medicinal plants as safer alternative therapies. Many plants have anxiolytic and/or antidepressant potential and contain diverse phytoconstituents which may be exploited for the development of new drugs to treat these disorders, particularly in cases where patients do not respond to current medications.[17,18] The present study was undertaken to investigate the anxiolytic and antidepressant activity of the methanol extract of *C. resiniferum* (MECR) leaves using behavioral models in mice. As reactive oxygen species play an important role in the pathophysiology of depression and anxiety,[19,20] we sought to further determine the levels of total phenolics and flavonoids in MECR and investigate the antioxidant/free-radical scavenging potential of this extract.

3. Materials & methods

3.1. Drugs and chemicals

Methanol (MeOH), ferric chloride ($FeCl_3$), aluminum chloride ($AlCl_3$), potassium ferricyanide, sodium carbonate (Na_2CO_3), potassium acetate and phosphate buffer were obtained from Merck (Darmstadt, Germany). Ascorbic acid (AC) and quercetin were obtained from BDH Chemicals Ltd. (Poole, UK). Gallic acid (GA), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), trichloro-acetic acid (TCA) and Folin-Ciocalteu reagent (FCR) were procured from Sigma Chemicals Co. (St. Louis, MO, USA). Diazepam and imipramine hydrochloride were purchased from Gonoshasthaya Pharmaceuticals Ltd (Dhaka, Bangladesh). All residual reagents were of analytical grade.

3.2. Plant material and extract preparation

The leaves of *Canarium resiniferum* (CR) were obtained from the Forest Research Institute, Chittagong, Bangladesh, in September 2019. The proper identification of the plant material was made by Prof. Dr. Shaikh Bokhtear Uddin, Herbarium Department of Botany, University of Chittagong, Bangladesh (accession number: CTGUH SR7925). Fresh and disease-free leaves were washed thoroughly and then left to dry naturally at 25 °C. The dried powdered leaves (500 g) were macerated in 100% MeOH (1.5 L) for 15 days with occasional shaking. Following filtration through cotton and Whatman no. 1 filter paper, the resulting solution was concentrated under reduced pressure to yield a gummy extract (3.4 g). An aliquot of this extract (10 g) was stored at 4 °C for further analysis.

3.3. Experimental animals

Swiss albino adult mice of both genders (each weighing ca. 23–30 g) were obtained from Jahangirnagar University, Dhaka, Bangladesh. The animals were acclimatized for a period of 14 days under controlled conditions (temperature: 25 ± 2 °C; relative humidity: 55–60%; 12 h light/dim cycle) and were given standard feed and water *ad libitum*. All tests were conducted from 9.00 a.m. to 5.00 p.m. Ethical approval for the investigation (Pharm-P&D-147/14–19/P153006) was obtained from by the Ethical Survey Panel and the P&D Board of the Department of Pharmacy, International Islamic University Chittagong, Bangladesh.

3.4. Experimental design

The mice were divided into groups (I -V) ($n = 6$) containing both male and female animals. Group- I was administrated the vehicle (1% Tween 80 in distilled water, p. o.). Group-II received the standard drug diazepam (1 mg/kg, i. p.) in the elevated plus-maze (EPM) test, the hole-board test (HBT), the light-dark box (LDB) test[21–24] and the standard drug imipramine (1 mg/kg, i. p.) - a tricyclic antidepressant - in the tail suspension test (TST) and the forced swim test (FST).[25,26] The remaining groups III, IV, V were given MECR (100, 200, 400 mg/kg, p. o.), respectively. These doses were selected based on the acute oral toxicity results and were similar to those reported in previous studies examining the anxiolytic and antidepressant of plant extracts.[22,27]

3.5. Acute oral toxicity study

The animals were separated randomly into 4 groups ($n = 6$) and were kept fasted overnight prior to the experiment. On the day of the experiment, the treated groups were administered MECR (1000, 2000, and 4000 mg/kg, p. o) while the control group received the vehicle orally. The mice were monitored for possible signs and symptoms of toxicity (e.g. sedation, allergic syndromes, motor impairment) over a short period (3 h) followed by a longer period (72 h). The mortality rate was recorded for each group up to 24 h after treatment.[28,29]

3.6. Evaluation of the anxiolytic activity

3.6.1. Elevated plus-maze (EPM) test

The EPM apparatus consisted of four arms, including two open (5 × 35 cm) and two closed (5 × 15 × 30 cm) arms, joint together with a central platform (5 × 5 cm). The maze was placed 60 cm above ground level. The animals from groups I–V were treated 30 min before the test was begun by placing individual animals on the central platform. The time spent in the open arms and the number of entries in the open arms were recorded over a period of 5 min.[30]

3.6.2. Hole-board test (HBT)

The hole-board (HB) test apparatus is a wooden compartment (40 × 40 × 25 cm) with 16 holes each 3 cm in diameter. The animals from groups I–V were treated 30 min before being placed individually on the HB apparatus. The number of head-dips was counted over a 5 min period of observation.[21]

3.6.3. Light-dark box (LDB) test

The LDB apparatus was a Plexiglas box with two compartments (each 25 × 25 cm) joint together. One of the compartments was dark and covered with a lid, the other one was brightly lit and open. The two compartments were connected by a 3 cm hole. The animals from groups I–V were treated 60 min before being placed individually in the light compartment of the apparatus and allowed to move around. The time that the animals spent in the light and the dark compartments was recorded for a period of 5 min.[22]

3.7. Evaluation of the antidepressant activity

3.7.1. Tail suspension test (TST)

Animals in group I–V were treated 30 min prior to being individually hanged 50 cm above the ground using adhesive tape placed about 1 cm from the tip of their tail and for a period of 6 min. The duration of immobility (in seconds) was recorded for the suspended animals within each group.[25]

3.7.2. Forced swim test (FST)

Animals in group I–V were treated 30 min prior to being placed individually for a period of 6 min inside a glass cylindrical chamber (25 cm high × 10 cm diameter) filled with water (up to 19 cm) at a temperature of 23 ± 1 °C. The duration of immobility (in seconds) of animals that stopped swimming was assessed during the last 4 min of the test.[26]

3.8. Statistical analysis

The results obtained from the behavioral tests were expressed as the means ± SEM of experiments run in triplicate. One-way analysis of variance (ANOVA), followed by Dunnett's multiple comparisons test, was used to analyse the differences between control and treated groups. P values < 0.05 were considered as statistically significant. All statistical analyses were performed using SPSS v. 16.0 and GraphPad Prism v 8.0 (GraphPad Software Inc., San Diego, CA).

3.9. Qualitative phytochemical analysis

MECR was subjected to a qualitative phytochemical analysis to identify phytoconstituents such as alkaloids, carbohydrates, proteins, glycosides, phenols, tannins, flavonoids and terpenoids as per standard protocols.[31]

3.10. Quantitative phytochemical analysis

3.10.1. Total phenolic content (TPC)

The total phenolic content of MECR was measured following a standard procedure.[32] An aliquot (0.5 mL) of MECR (1 mg/mL) was mixed with 2.5 mL of FCR (10%, w/v) and 2 mL of Na_2CO_3 (7.5%, w/v). The mixture was incubated for 5 min at 50 °C and then left to cooled down. The absorbance was measured at 760 nm against distilled water as a blank. A standard calibration curve was generated using six concentrations of gallic acid (15.62–500 μg/mL) and TPC was expressed as mg of gallic acid equivalents (GAEs) per g of dried MECR. The test was performed in triplicate.

3.10.2. Total flavonoid content (TFC)

The total flavonoid content in MECR was determined using a colorimetric assay.[33] Aluminum chloride (10% w/v, 0.2 mL), potassium acetate (1 M, 0.2 mL), MeOH (3 mL) and distilled water (5.6 mL) were added to either 1 mL of MECR (1 mg/mL) or quercetin (12.5–100 μg/mL). The resulting mixture was incubated for 30 min at 25 °C and absorbance was measured in a spectrophotometer at 420 nm against distilled water as a blank. The flavonoid content was expressed as mg of quercetin equivalents (QEs) per g of dried MECR. The test was performed in triplicate.

3.11. Determination of the antioxidant effect

3.11.1. DPPH radical scavenging assay

The DPPH assay was carried out according to a previously published protocol.[34] DPPH (0.004%, w/v) (3 mL) was added to MeOH (3 mL) and various concentrations (500–15.625 μg/mL) of MECR. The resulting mixture was left at 25 °C for 30 min, and absorbance was measured in a spectrophotometer at 517 nm against distilled water as a blank. Ascorbic acid (500–15.625 μg/mL) was used as a positive control. The test was performed in triplicate. The percentage of radical scavenging activity was calculated using the following equation:

$$\text{Scavenging } \% = (\text{Abs}_{\textbf{control}} - \text{Abs}_{\textbf{sample}})/\text{Abs}_{\textbf{control}}] \times 100$$

where $\text{Abs}_{\textbf{control}}$ = Only DPPH solution and $\text{ABS}_{\textbf{sample}}$ = sample (extract or standard) + DPPH solution.

3.11.2. Ferric reducing antioxidant power (FRAP) assay

The reducing power capacity of MECR was evaluated using a previously described methodology.[35] MECR (1 mL) was sequentially mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%, w/v). This mixture was incubated for 20 min at 50 °C and then mixed with 2.5 mL of trichloroacetic acid (10%, v/v). Following centrifugation for 10 min at 3000 rpm, the upper solution (2.5 mL) was transferred to a test tube and was subsequently mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride (0.1%, w/v). The absorbance was measured in a spectrophotometer at 700 nm against distilled water as a blank. Ascorbic acid (15.62–500 μg/mL) was used as a positive control. The FRAP values were expressed as content of Fe(II) in μM/mg of extract using a standard curve with different concentrations of FeSO4. The test was performed in triplicate.

4. Results

4.1. Acute oral toxicity study

Neither lethal effects nor evidence of behavioral toxicity (i.e. defecation, urination, lacrimation, salivation, pilo-erection, aggressiveness, overactivity, convulsions, tremors, twitches) were

observed in animals following the oral administration of MECR at doses of 1000, 2000, and 4000 mg/kg. Therefore, MECR was deemed to be safe even at the highest dose level of 4000 mg/kg, and its lethal dose (LD_{50}) was considered be > 4000 mg/kg. On that basis, the doses of extract (100, 200 and 400 mg/kg) chosen for the subsequent in vivo experiments were considered as safe.

4.2. Evaluation of the anxiolytic activity

4.2.1. Effects of MECR in the elevated plus maze (EPM) test

The effects of MECR on the time spent and the number of entries in the open arms in the EPM test are illustrated in Fig. 1. Administration of MECR (100, 200, and 400 mg/kg) showed anxiolytic activity by increasing both the time spent and the number of entries in the open arms in a dose-dependent manner. The time spent and the number of entries in the open arms were significantly increased in groups treated with MECR at 200 and 400 mg/kg. At 200 mg/kg, MECR showed a moderate but significant anxiolytic effect in both the time spent (75 ± 2.98 s; $P < 0.001$) and the number of entries (10.33 ± 0.92; $P < 0.01$). At 400 mg/kg, it greatly increased the time spent (97.33 ± 4.22 s; $P < 0.001$) and the number of entries (18.83 ± 1.40; $P < 0.001$). Mice treated with 100 mg/kg did not manifest significant improvement ($P > 0.05$) in the time spent and number of entries in the open arms. As expected, diazepam at 1 mg/kg (positive control) significantly raised the time spent (93 ± 2.42 s; $P < 0.001$ vs. control group) and number of entries (20 ± 0.82; $P < 0.001$) in the open arms.

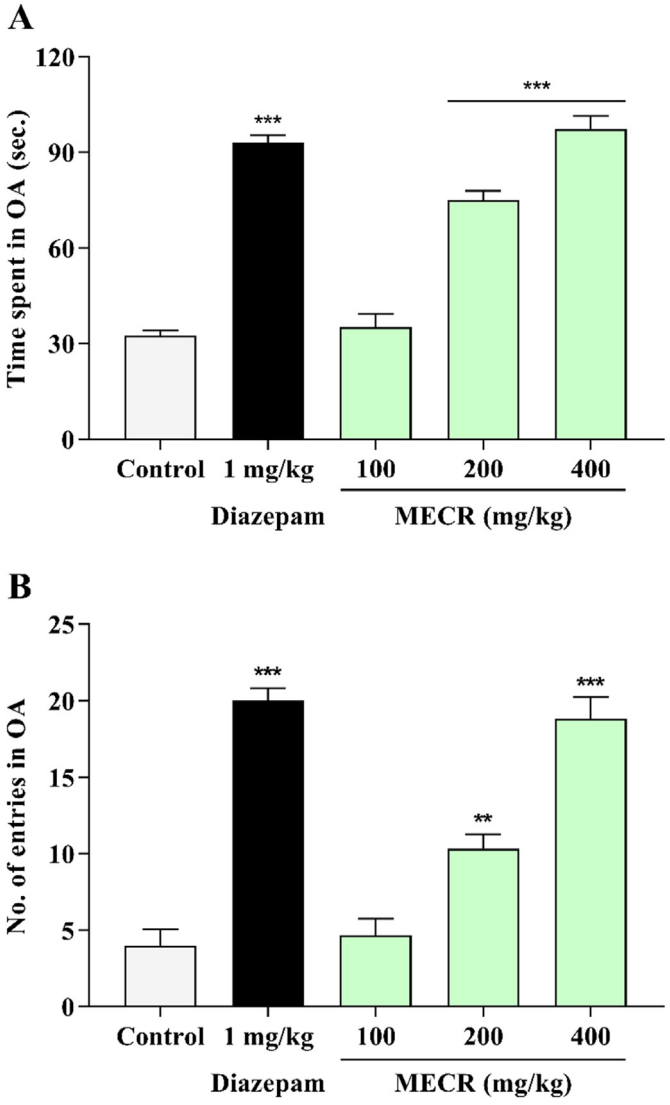

Fig. 1. Effects of MECR (100, 200, 400 mg/kg, p. o.) and diazepam (1 mg/kg, p. o.) on the (**A**) time spent in the open arms (in seconds) and (**B**) number of entries in the open arms in the EPM test. Values are presented as means ± SEM ($n = 6$). The data sets were analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test. $^{**}P < 0.01$, and $^{***}P < 0.001$ were considered significant as compared to the control. MECR, methanol extract of *C. resiniferum* leaves.

4.2.2. Effects of MECR in the hole-board test (HBT)

The effects of MECR on the head-dip counts in the HBT are illustrated in Fig. 2A. MECR (at doses of 400 and 200 mg/kg) significantly and dose-dependently increased the number of head-dips by 111.43% (37 ± 2.19; $P < 0.001$) and 58.01% (27.67 ± 2.56; $P < 0.01$), respectively compared to the control group. The extract at a dose of 400 mg/kg showed a head-dip count and % head-dips increase superior to that of the positive control diazepam at 1 mg/kg (34.33 ± 1.33; 96.19%). No significant effect was recorded at the dose of 100 mg/kg.

4.2.3. Effects of MECR in the light-dark box (LDB) test

The effects of MECR on the time spent in the light and the dark compartments in the LDB test are illustrated in Fig. 2B. When compared to the control, MECR at 400 and 200 mg/kg significantly ($P < 0.001$) increased the time spent in the light box (173.05 ± 8.11 and 128.27 ± 4.34 s, respectively) and significantly ($P < 0.001$) decreased the time spent in the dark box (126.95 ± 8.11 and 191.73 ± 4.34 s, respectively). The group of animals treated with 100 mg/kg did not manifest a significant increase/decrease ($P > 0.05$) in the time spent in the light/dark box. In the standard drug diazepam-treated group (1 mg/kg), the time spent in the light and dark box were 177.57 ± 5.64 and 122.43 ± 5.64 s, respectively (P < 0.001 vs. control group). The values for the time spent in the light/dark box observed for MECR (400 mg/kg) were comparable to those obtained after administration of the standard drug diazepam.

4.3. Evaluation of the antidepressant activity

4.3.1. Effects of MECR in the tail suspension test (TST) and the forced swim test (FST)

The effects of MECR on the duration of immobility in the TST and FST are illustrated in Fig. 3A and B, respectively. In both tests, MECR (100,200,400 mg/kg) dose-dependently reduced the duration of immobility compared to untreated animals (control) and this was significant ($P < 0.001$ vs control) for all doses, except for the dose of 100 mg/kg in the FST model. The standard drug imipramine (1 mg/kg) showed a significant reduction in the duration of immobility (P < 0.001 vs control), and the effect of MECR at the highest dose of 400 mg/kg was comparable to that of imipramine in both tests.

4.4. Qualitative phytochemical analysis

Preliminary phytochemical profiling of MECR revealed the presence of alkaloids, carbohydrates, proteins, phenols, tannins, and flavonoids (Table 1).

4.5. Determination of TPC, TFC, antioxidant activity

The total phenolic and flavonoid content of MECR were determined as 90.94 ± 0.75 mg GAE/g and 51.54 ± 0.78 mg QE/g of dried extract, respectively (Table 2).

In the DPPH assay, MECR showed concentration-dependent radical scavenging activity, with the maximum percentage

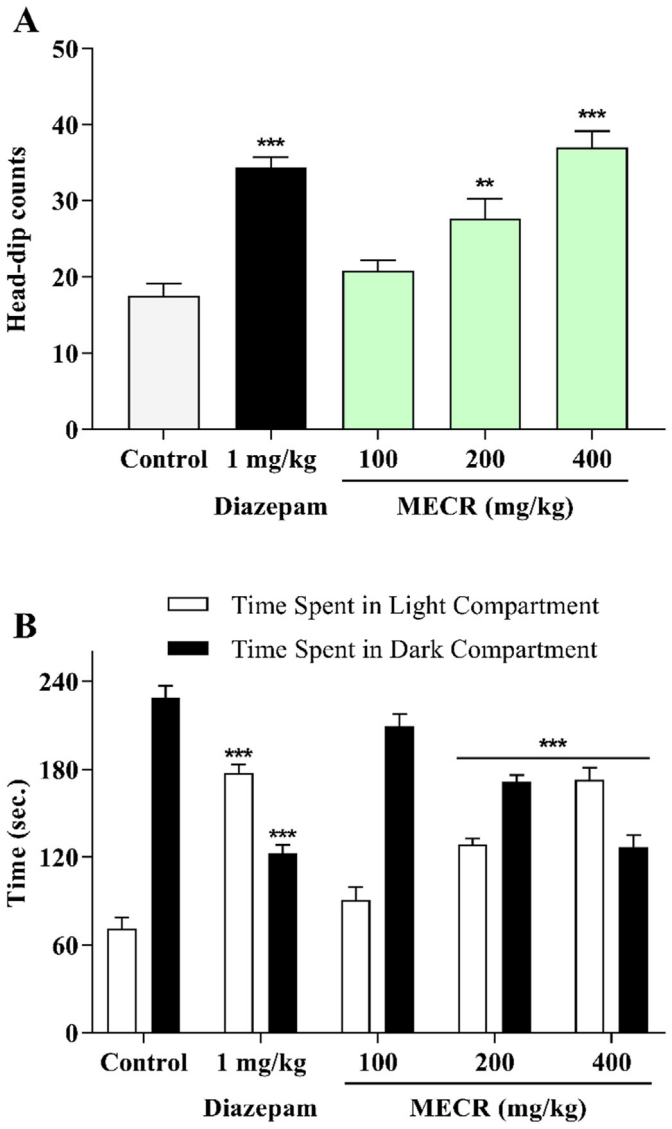

Fig. 2. Effects of MECR (100, 200, 400 mg/kg, p. o.) and diazepam (1 mg/kg, p. o.) (**A**) on the head-dip counts in the HBT and (**B**) the time spent in the light and the dark compartments in the LDB test. Values are presented as means ± SEM ($n = 6$) along with the mean % increase in head-dip counts. The data sets were analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test. $^{**}P < 0.01$, and $^{***}P < 0.001$ were considered significant as compared to the control. MECR, methanol extract of *C. resiniferum* leaves.

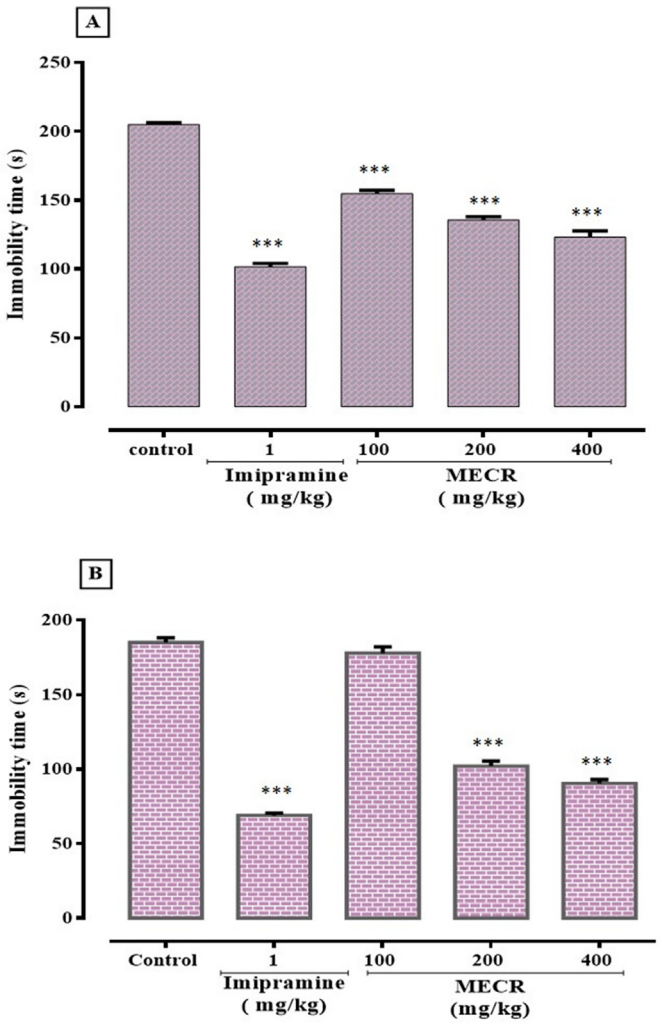

Fig. 3. Effects of MECR (100, 200, 400 mg/kg, p. o.) and imipramine (1 mg/kg, i. p.) on the duration of immobility (**A**) in the TST and (**B**) in the FST. Values are presented as means ± SEM ($n = 6$). The data sets were analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test. $^{***}P < 0.001$ was considered significant as compared to the control. MECR, methanol extract of *C. resiniferum* leaves.

(62.44%) recorded at the highest concentration (500 μg/mL). The IC_{50} value obtained for MECR (177.82 ± 2.77 μg/mL) was higher than that of the standard ascorbic acid (IC_{50} value of 25.62 ± 0.68 μg/mL) (Fig. 4A). In the ferric reducing antioxidant power (FRAP) assay, MECR demonstrated concentration-dependent reducing capability compared to ascorbic acid. At 1000 μg/mL, the absorbance of MECR and ascorbic acid were 0.969 and 3.251, respectively (Fig. 4B).

5. Discussion

Anxiety and depressive disorders are common mental disorders that are often experienced simultaneously and that can severely impair the quality of life of sufferers.[14] In this study, the effects of MECR on anxiety-related behavior were assessed in mice using the EPM, HBT, and LDB tests; three behavioral models widely used to investigate the anxiolytic potential of drugs, including plant-based ones.[36,37] The EPM test is one of the most commonly used animal models for testing anxiety-related behavior. It is based on the fact that elevated and open sections of the maze trigger fear and anxiety in rodents which in turn tend to avoid spending time in these places and prefer safer (closed arm) sections. Treatment with an

Table 1
Preliminary phytochemical screening of MECR.

Phytoconstituents	Test performed	Observations
Alkaloids	Mayer's test	+
	Wagner's test	+
Carbohydrates	Benedict's test	+
	Molisch's test	+
Proteins	Biuret test	+
Glycosides	Borntrager's test	-
Phenols	Ferric Chloride test	+
Tannins	Gelatin test	+
Flavonoids	Alkaline Reagent test	+
Terpenoids	Salkowski test	-

+/− sign indicates presence/absence of the phytoconstituent.

Table 2
Total phenolic content (TPC), total flavonoid content (TPC), and radical scavenging activity of MECR.

	TPC (GAE in mg/g of dry extract)	TFC (mg QE/g of dry extract)	DPPH Assay IC_{50} (μg/mL)
MECR	90.94 ± 0.75	51.54 ± 0.78	177.82 ± 2.77
AA	-	-	25.62 ± 0.68

Values are expressed as mean ± SEM ($n = 3$). MECR, methanol extract of *C. resiniferum* leaves; AA, ascorbic acid; GAE, gallic acid equivalent; QE, quercetin equivalent; DPPH, 2,2-diphenyl-1-picrylhydrazyl; -: not assessed.

anxiolytic agent encourages exploratory behavior and increases the time spent and the number of entries in the open arms of the maze.[38] In the present study, MECR (200 and 400 mg/kg) as well as diazepam (1 mg/kg) showed significant anxiolytic activity by increasing both the time spent and the number of entries of treated animals in the open arms. In the HBD test, the degree of anxiety in animals is assessed by observing head-dipping behavior, with anxiolytic drugs triggering an increase in the head-dip counts.[23] In this study, MECR (at doses of 400 and 200 mg/kg) significantly increased the number of head-dips compared to the control group, and at 400 mg/kg showed a head-dip count and % head-dips increase superior to that of the standard drug diazepam. The anxiolytic effect of MECR was further investigated using the LDB test. The LDB apparatus comprises of a dark (safe) compartment and a bright (aversive) compartment. The LDB test relies on the inherent aversion of rodents to bright areas, with anxiolytic drugs increasing the time spent by animals in the light compartment rather than the dark one.[24] MECR at 400 and 200 mg/kg significantly increased the time spent in the light box and significantly decreased the time spent in the dark box. The effects observed for MECR at 400 mg/kg were comparable to those obtained after administration of diazepam. MECR at the dose of 400 mg/kg only showed significant anxiolytic activity compared to untreated animals in all three tests.

The antidepressant activity of MECR was evaluated in mice using the well-established TST and FST behavioral models. When animals are placed under stressful conditions (i.e. inescapable positions), they tend to remain immobile for a long duration. This state of immobility is a reflection of an inability to adjust to the stressful situation, despair or loss of hope to be able to escape. Such behavior closely resembles what is observed in depression.[39,40] Treatment with an antidepressant leads to a decrease in the duration of immobility. In the present study, MECR significantly reduced the duration of immobility compared to untreated animals for all doses, except for the dose of 100 mg/kg in the FST model. The effect of MECR at 400 mg/kg was comparable to that of the standard drug imipramine in both tests.

Several mechanisms have been proposed to explain the pathogenesis of anxiety and depression. The latter has been linked to a dysregulation of the neurotransmitter systems (mainly serotonin and norepinephrine) in the CNS, with antidepressants like selective serotonin reuptake inhibitors (SSRIs) and serotonin and noradrenaline reuptake inhibitors (SNRIs) inhibiting the re-uptake of these neurotransmitters, and monoamine oxidase inhibitors (MAOIs) inhibiting their degradation.[41–44] It has also been linked with excessive activation of the hypothalamic-pituitary-adrenal axis which stimulates neurons to discharge the stress-related neuropeptide corticotropin-releasing factor.[45] Other, more recent, studies have highlighted the role of oxidative stress/damage in depression as well as anxiety disorders.[19,20] Natural products such as polyphenols and flavonoids are well-known for their free radical scavenging/antioxidant activity.[46,47] The DPPH and the FRAP assays are two colorimetric *in vitro* tests that are commonly employed to measure the free-radical scavenging activity and the reducing power of antioxidant drugs, respectively.[48] In the present investigation, MECR showed high radical scavenging activity in the DPPH assay as well as some ferric reducing antioxidant power. This may be attributable to the high total phenolic and total flavonoid contents of MECR.

Qualitative phytochemical analysis showed that MECR contained a range of structurally-diverse secondary metabolites, including alkaloids, tannins, phenols, and flavonoids. Previous studies have demonstrated that alkaloids, flavonoids, and phenols had anxiolytic activity owing to their high affinity for the benzodiazepine (BZD)-binding site of $GABA_A$ receptors.[49] Gamma-aminobutyric acid (GABA) is an important inhibitory neurotransmitter in the central nervous system (CNS). The binding of BZDs to $GABA_A$ receptors increases the opening of the linked chloride channel, leading to neuronal membrane hyperpolarization and

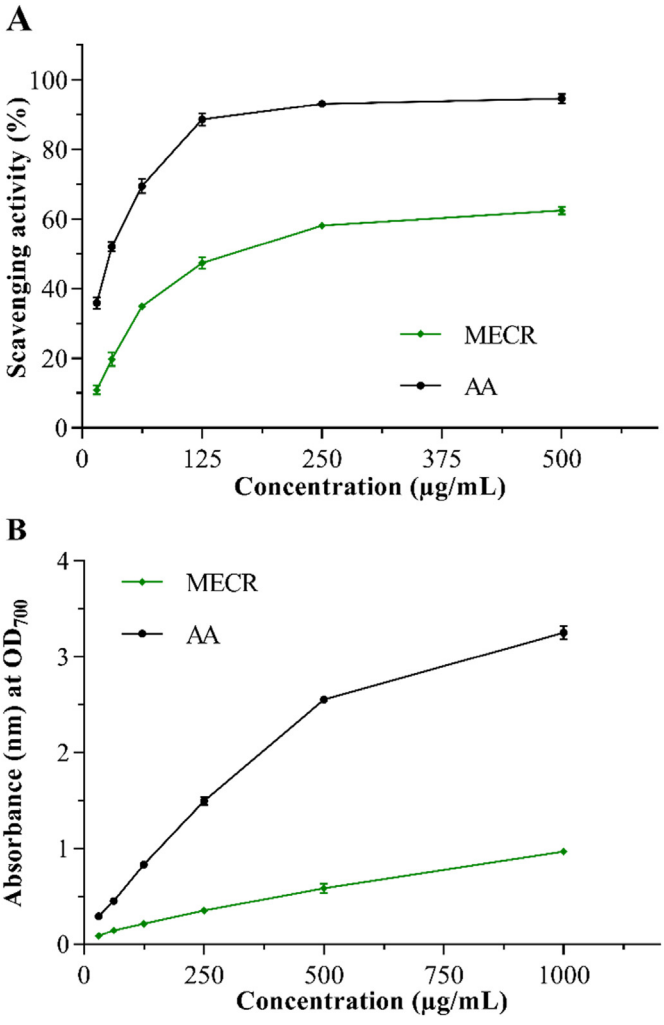

Fig. 4. Antioxidant activity of MECR in the DPPH free radical scavenging and ferric reducing antioxidant power (FRAP) assay. (**A**) % DPPH free radical scavenging activity of MECR and ascorbic acid at different concentrations. (**B**) Ferric reducing power capacity of MECR and ascorbic acid at different concentrations. Values are presented as means ± SEM ($n = 3$). MECR, methanol extract of *C. resiniferum* leaves; AA, ascorbic acid.

anxiolytic activity.[50] Other studies reported that plants rich in total phenolics and tannins could exert beneficial effects in anxiety and depression via upregulating the expression of $GABA_A$ and 5-HT_{1A} receptors, serotonin, norepinephrine, dopamine, brain-derived neurotrophic factor, cAMP response element-binding protein, and reducing serum cortisol levels in animals.[51,52]

6. Conclusion

The above results revealed the lack of acute oral toxicity (up to a dose of 4000 mg/kg) and significant anxiolytic and antidepressant activity (at a dose of 400 mg/kg) of the methanol extract of *C. resiniferum* leaves in mice. They also showed that this extract was rich in phenolic compounds, including flavonoids, and possessed a high free radical scavenging effect *in vitro*. This suggests that *C. resiniferum* leaves may represent an alternative treatment for anxiety and depressive disorders at a human equivalent dose (HED) of 32.5 mg/kg, and one that would be safe (up to HED 325 mg/kg).[53] Further investigations are warranted to link the anxiolytic/antidepressant activity of MECR to the presence of individual bioactive phytoconstituent(s). The antioxidant phenolics/flavonoids, or other phytoconstituents with a separate mechanism of action, in MECR may serve as templates for the development of new treatments for depression and/or anxiety in the future.

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Declaration of competing interest

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