Evaluation of antidepressant and nootropic activities of leaf extracts of *Rhizophora apiculata*

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Background
Rhizophora (R.) apiculata is a traditional mangrove plant having antioxidant, anti-inflammatory, and central analgesic activities.
Objective
The current study was performed to assess the beneficial neurological activities of the plant using rodent models and also to explore the phytochemical distribution of plant extracts using the hyphenated analytical technique.
Materials and methods
Ethyl alcohol and aqueous extracts were subjected to phytochemical screening, followed by GC-MS analyses. In experimental studies, the animals were divided into normal, positive control (standard), negative control, and extract-treated groups at three doses of each extract. The tail suspension method and forced swim tests were used as requisite animal models for the evaluation of antidepressant activity. Imipramine was used as the standard drug for the evaluation of antidepressant studies. Nootropic activity was evaluated by using the radial arm maze and Y-maze models. For these studies, scopolamine was used to impair the cognition of the animals and donepezil was used as the standard drug. The results were displayed as mean±standard error mean, and two-way ANOVA was used to analyze statistical significance between the test groups.
Results and conclusion
Preliminary phytochemical analyses showed that the leaves contain a wide range of secondary metabolites in abundance. As per GC-MS characterization, a few bioactive compounds like 3-O-methyl-D-glucose, desulphosinigrin, 1,25-dihdroxy vitamin D3, and ethyl iso-allocholate were identified. Ethyl alcohol extract (at 300 mg/kg; and 600 mg/kg) and aqueous extract (at 200 mg/kg; and 400 mg/kg) of *R. apiculata* exhibited antidepressant activity in both models. The plant extracts were proved to have cognition-enhancing activities at tested doses. The results stated that the plant *R. apiculata* is proved to have antidepressant and cognition-enhancing activities. Thus, it may provide a chance in the therapeutic management of neurological ailments. The effects of leaf constituents on brain neurotransmitter levels and the histology of the brain need to be established by future investigations.

Keywords:
antidepressant activity, flavonoid content, GC-MS study, nootropic activity, *Rhizophora apiculata*

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Introduction

Depression is a serious psychological disorder related to the brain, which is characterized by severe sadness, dysphoria, insomnia, anorexia and deprivation of interest in all pleasures. Symptoms of depression may include intense sadness, mood swings, irritability, insomnia, and social distancing. Depression may be caused by the deprivation of monoamine neurotransmitters at the synapse [1]. Tricyclic antidepressants and monoamine oxidases are the major drugs that are used in the current treatment. The majority of these medications have severe adverse effects as they are synthetic in nature. Hence, this is a small approach to derive active molecules from natural sources for the treatment of depression and to improve the cognitive skills of those who are suffering from neurodegenerative disorders.

Cognition enhancers are the drugs that are used in the management of cognition deficiency in the elderly with Alzheimer's disease, stroke, schizophrenia, Parkinson's disease, and cerebral palsy [2]. The reduced levels of neurotransmitters like Acetylcholine (Ach) are mainly associated with reduced cognition in chronic diseases. Few plants with antidepressant activity like

Convolvulus pluricaulis, Evolvulus alsinoides, and Clitoria ternate have proven to have memory-enhancing effects [3].

Mangrove ecosystems are distinct and are significant for humans in numerous ways [4]. The local coastal community uses a variety of therapeutic species found in mangrove forests to cure a variety of health issues, including neurological illnesses, diabetes, cardiac disorders, and hepatic disorders [5]. *Rhizophora (R.) apiculata* is a marine mangrove plant that is found along the coasts of subtropical and tropical regions of the world [6], including Singapore, India, Pakistan, and other regions. Ponna, or uppu ponna, is the local name for this plant. *R. apiculata* is an Indian mangrove plant with a wide range of medical applications, including anti-inflammatory, antidiabetic, wound-healing, antioxidant, and anticancer properties [7]. In our earlier study, the plant extracts possessed central analgesic activities. These investigations were continued to determine its nootropic activity [8]. Therefore, the current study began with a phytochemical screening and then evaluated the antidepressant [9] and nootropic [10] activities.

Materials and methods

Plant material

The plant was identified and collected from the coastal mangrove woods in the Guntur district, Andhra Pradesh, India, and authenticated by a taxonomist (Dr. Raghu Ram, Department of Botany at Acharya Nagarjuna University) using the voucher specimen (RAR/7-2(872)/2019/ANU/BOT). The leaves were picked from the plant, cleansed with a dry cloth, and dried for 7 days in the shade as well as in a hot air oven set at 45°C. The dried leaves were ground into a fine powder.

Chemicals and drugs

The chemicals and solvents used in this study were purchased from SD Fine chemicals, Thermo Fisher Scientific India Pvt. Ltd, and Merck India. The pure form of Imipramine was obtained from Healthy Inc Mumbai. Scopolamine was purchased from Mylan Pharmaceuticals Inc. and donepezil was purchased from Cipla Ltd, Mumbai.

Preparation of the crude extract

The leaf powder was successively extracted with diethyl ether, ethyl alcohol, and water in increasing order of polarity, from which the crude extract was obtained. By macerating the material for three days, diethyl ether extract was produced. Soxhlet extraction was used for continuous extraction until the clear solvent was recovered to obtain the ethyl alcohol extract of *R. apiculata* (EERA) and aqueous extract of *R. apiculata* (AERA). To dispose of excess solvents, the extract was strained through Whatman filter paper and dried. The resultant extract was weighed and placed in a refrigerator.

Phytochemical study

In accordance with the standard phytochemical screening procedures described by Khandelwal *et al.*, both plant extracts were screened for the presence of pharmacologically active phytochemical components such as alkaloids, carbohydrates, flavonoids, glycosides, phenols, saponins, tannins, and triterpenoids [11].

Total flavonoid content

Quercetin was used as a standard to determine the total flavonoid composition of the two extracts using aluminum chloride ($AlCl_3$) test. In this method, 1 ml of the test sample was mixed with 4 ml of water in a 10 ml of volumetric flask. After 5 min, sodium nitrate (5%, 0.3 ml) and $AlCl_3$ (10%, 0.3 ml) were added to it and kept aside for 10 min at room temperature. To this reaction mixture, 1 ml of sodium hydroxide (1M) was added, and the volume was adjusted to 10 ml with distilled water. Using an ultraviolet (UV)-visible spectrophotometer, the absorbance of the sample was determined at 510 nm. The same experiment was repeated in triplicate for precision, and the results were expressed as mean ±standard error mean (SEM) in terms of the flavonoid content (quercetin equivalents per gm of dry weight) [12].

GC-MS study

The sample was diluted with 500 µl of *n*-hexane, vortexed for 1 min, and then placed into a glass gas chromatography vial with a screw cap. Analyses of the sample's metabolite profile were performed using gas chromatography-mass spectrometry (GC-MS) with an electron impact ionization (EI+) source operating at 70 eV (GC 7890 and MS of 5977 N, Agilent Technologies, Palo Alto, CA, USA). An aliquot of 1 µl of the extract was injected into the injector port (at 250°C) to conduct the analysis. Helium was used as the carrier gas throughout the analysis, flowing at a rate of 1 ml/min. An HP-5 MS capillary column (30 m 250 µm i.d. 0.25 µm) with a stationary phase made of 5% phenyl and 95% methylpolysiloxane in splitless mode was utilized for the analysis. The GC oven temperature program had the temperature set out at 65°C for 2 min, ramp up to 230°C at 6°C/min, and

then increased to 290°C at 10°C/min, where it was kept for 20 min. The ion source was set to be at 230°C, and the interface temperature was set at 290°C [13].

Experimental animals

Healthy, male, adult albino mice that weigh about 27 ±5 g of age 9–12 weeks were picked out randomly and were maintained at standard conditions (25°C±2°C and 50%±20% humidity) with 12 h day and night cycles. Weights of all the animals were recorded, and the animals were accustomed for 5 days before the experimentation period. A commercial pellet diet and water were provided throughout the duration.

Ethical approval

The present study was carried out following approval from the Institutional Animal Ethics Committee (IAEC), Bapatla College of Pharmacy, Bapatla, Andhra Pradesh with reference number IAEC/XIV/06/BCOP/2021.

Preparation of samples

The 0.1% carboxy methyl cellulose (CMC) was used as the vehicle for the EERA, while distilled water was used as the vehicle for the AERA.

Antidepressant activity

Antidepressant activity was assessed by using the forced swim test (FST) and tail suspension test (TST). Animals were randomly divided into different groups, containing six animals in each group. The control group was given 1 ml of saline. The positive control group was given the standard drug. The remaining groups received test samples of EERA (150, 300, and 600 mg/kg dose) and AERA (100, 200, and 400 mg/kg dose). The doses were selected based on the toxic profile and effective doses of earlier acute toxicity studies [7].

The animals were divided into eight groups, where group I serves as the normal control and takes distilled water, group II serves as the positive control takes the standard drug Imipramine 15 mg/kg; groups III, IV, and V have received EERA at three doses of 150 mg/kg, 300 mg/kg, and 600 mg/kg, respectively, and groups VI, VII, and VIII received AERA at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg respectively. All the drugs are administered by the oral route.

Forced swim test (FST)

In this test, the animals are forced to swim in an inescapable container having water; in the process of escape trials, the animals develop a stress response as a result of the neural limb pathway. Such stress results in depression-like symptoms. During experimentation, the mice were kept individually in separate beakers containing water up to a height of 15 cm at room temperature. Then the animals were allowed to swim and the duration of immobility was recorded with a total duration of 6 min. The immobility of the animal means it is motionless or it is doing only a few movements to keep its head above the surface of the water. Forced swim tests were used to evaluate different antidepressant agents [14].

Tail suspension test (TST)

In this test, the animals were subjected to unavoidable stress by hanging them by their tails, which cause them depressive symptoms like immobile posture and a decrease in escaping tendency. Tail suspension tests are intended to evaluate the potency of various antidepressant agents. This test is based on the principle to measure the duration of immobility, which indicates a state of despair when subjected to unavoidable stress, which reflects human depressive disorders. In this model, the animals were suspended from a platform at a height of 50 cm above the ground with the help of adhesive tape, which is placed 1 cm above the tip of the tail. The duration of immobility was recorded for the last 4 min for a total of 6 min of suspending time [15].

Nootropic activity

The radial arm maze and Y-maze were used to assess he *R. apiculata* leaf extract's predicted nootropic efficacy. The experiment protocol includes nine groups of animals with six animals in each group; the first group serves as the normal control, which receives only distilled water, orally. The second group serves as a positive control, which receives an inducing agent and standard drug. Here, scopolamine (ip, 20 mg/kg) was used as an inducing agent and donepezil 2.5 mg/kg (oral) was used as the standard drug to treat amnesia induced by scopolamine. The third group is regarded as the negative control which receives only the inducing agent (Scopolamine 20 mg/kg). The next three groups (4, 5 and 6) were administered with scopolamine 20 mg/kg and EERA at three doses (150, 300, and 600 mg/kg). The remaining three groups (7, 8, and 9) have received scopolamine (20 mg/kg) and AERA at three doses (100, 200, and 400 mg/kg). All the extracts were administered through the oral route and the inducing agent was administered through the intraperitoneal route.

Radial arm maze test

The radial arm maze consists of 8 arms numbered as 1–8 radiating from the center. The equipment was placed 40 cm above the ground. By the end of each arm,

there was a food cup that had a single food cup. The animals were kept on a restricted diet before the 8 h of the experimentation period. Before start of the experiment, the rats were habituated to the maze by placing pellets throughout the maze and were allowed to move freely and explore the food. The next day the food was restricted only to the food cups. From day 3 to day 6, the food was placed only at any one of the arm food cups 40 min after the administration of the test and standard. The rats were individually placed into the radial arm maze and were observed for 10 min or for 16 arm entries or until all pellets have been eaten. In this experiment, the loss of memory was induced by Scopolamine (20 mg/kg) due to which the animals repeatedly enter the same arm, which is not having food pellets and these entries can be regarded as errors. Reference error mean, spatial working memory error, and transfer latency times were calculated as parameters to measure the nootropic potential of the extract [16].

Y-maze test

Y-maze method is a simple way to evaluate the memory-enhancing activity. The wooden Y-maze consists of three arms at an angle of 1200 degree in between the arms. Each arm is 30 cm in length, 8 cm in width, and 15 cm in depth. On day 1, the animals were habituated to the maze for 10 min by scattering food pellets on the arms of the maze. On the next day, the rats were allowed to explore the maze arms sequentially in the order of A, B, and C by placing the pellets. Variations in these sequential entries were regarded as alterations. The animals whose memory was lost by the inducing agent (scopolamine) showed more alterations. Visually, 13 entries were recorded for every rat and the percentage (%) of spontaneous alterations was calculated by using the following formula [17]:

$$\%\text{Number of alterations} = \text{Number of actual alterations}/\text{No. of possible trials} * 100$$

Statistical analysis

Data analysis was performed using GraphPad Prism version 9.5, and the results were presented as mean ±SEM. The significance of the results was verified by computing *P* values using two-way analysis of variance (ANOVA). In addition, error bars were displayed to show the results from our animal experiments' standard error mean.

Results and discussions

Phytochemical studies

Preliminary phytochemical investigations of EERA and AERA extracts were found to be comprised of flavonoids, carbohydrates, tannins, glycosides, and alkaloids [7].

Total flavonoid content

The extracts EERA and AERA were observed to have 76±0.34%, 52±0.87% of flavonoid contents, respectively.

GC-MS study

The GC-MS analysis of EERA and AERA indicated the various components corresponding to the bioactive substances relating to the known compounds described by the NIST library. *N*-(*N*-glycyl-leucyl)-glycine, 3-O-methyl-d-glucose, thymol, desulphosinigrin, 1, 25-dihydroxyvitamin D3, ethyl iso-allocholate, squalene, and methyl glycocholate were commonly seen in both extracts. In addition, the AERA also comprises melezitose, phytol, β-amyrin, lupeol, and DL-tocopherol. Among these, 1,25-dihydroxy vitamin D3 possesses anti-inflammatory properties against interleukin-induced arthritic inflammation and can lessen oxidative stress [18]. Also, it can promote the development of neural stem cells into oligodendrocytes [19], which may have a positive impact on neuronal plasticity. In addition, several bioactive compounds were identified, which possess a variety of antifungal [20], antiparasitic, hepatoprotective [21], antitumor [22], anti-inflammatory [23], cytotoxic [24], antibacterial, and antioxidant activities [25]. The reported biological activities of compounds of EERA and AERA are listed in Tables 1 and 2.

Antidepressant activity

The effect of EERA and AERA extracts on FST is illustrated in Fig. 1. The EERA treatment has demonstrated decreased immobility time when compared with pretreatment groups with increasing doses as per the FST. In this test, EERA treatment at 150 mg/kg and AERA 100 mg/kg dosage did not show a significant decrease in the immobility time. However, the EERA (300 mg/kg and 600 mg/kg) and AERA 400 mg/kg has shown a significant decrease in the immobility time.

In the TST test (Fig. 2), EERA 600 mg/kg and AERA 200 mg/kg and 400 mg/kg doses have shown considerable reduction in the immobility time when compared with the pretreatment group with a significant *P* value ($P<0.001$).

Nootropic activity

In Figs. 3 and 4, the results of the radial arm maze test are displayed statistically. The nootropic activity was investigated in terms of cognition and memory-

Table 1 Phytochemicals detected in EERA as per the GC-MS report

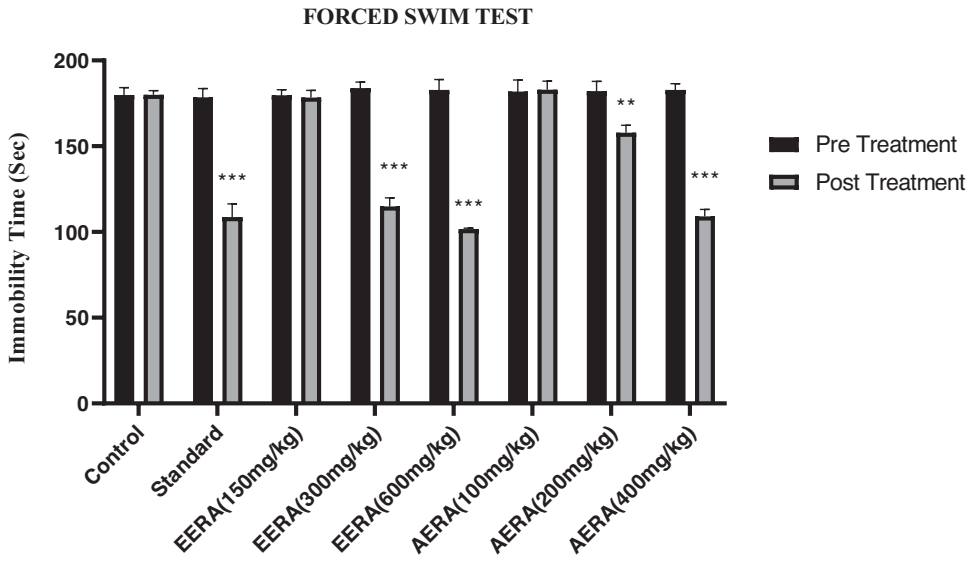
Rt (min)	Compound name	MF	MW (gm/mol)	Pharmacological activities
7.697	Cyclohexyldimethoxymethyl silane	$C_9H_{20}O_2Si$	188.34	None
12.080	1,25-dihydroxy vitamin D3	$C_{27}H_{44}O_3$	416.6	Calcium channel agonist, antirachitic, antipsoriatic agent[26,27]
13.418	2-Bromotetrad-ecanoic acid	$C_{14}H_{27}BrO_2$	307.27	Antifungal activity[28]
14.696	Desulphosinigrin	$C_{10}H_{17}NO_6S$	279.31	Antiparasitic activity[20]
16.132	3-O-methyl-d-glucose	$C_7H_{14}O_6$	194.18	Contrasting agent in tumor detection[29]
17.295	n-Hexadecenoic acid	$C_{16}H_{32}O_2$	256.42	Anti-HPV agent, antiapoptotic agent[30]
19.767	*N*-(*N*-glycyl-leucyl)-glycine	$C_{10}H_{19}N_3O_4$	245.28	None
21.437	Hexadecamethylheptasiloxane	$C_{16}H_{48}O_6Si_7$	533.1	None
22.067	Methyl glycocholate	$C_{36}H_{69}NO_6Si_3$	696.2	None
22.124	Ethyl-iso-Allocholate	$C_{26}H_{44}O_5$	436.6	Anti-inflammatory agent, anti SARS-Cov 2 virus[23,31]
26.122	Octa siloxane	O_7Si_8	336.68	None
24.744	Squalene	$C_{30}H_{50}$	10.7	Adjunctive and protective agent in chemotherapy[32]

Table 2 Phytochemicals detected in AERA as per the GC-MS report.

Rt (min)	Compound name	MF	MW (gm/mol)	Pharmacological activities
8.107	Dodecene	$C_{12}H_{24}$	168.32	None
8.710	D-Glucose, cyclic 1,2-ethanediyl mercaptal	$C_8H_{16}O_5S_2$	256.3	None
8.995	Silane, dodecyl diethoxy methyl-	$C_{17}H_{38}O_2Si$	302.6	Adhesive in dental and surface treatments [32]
9.576	Thymol	$C_{10}H_{14}O$	150.22	Antibacterial and antifungal activity [33]
10.850	3-Trifluoroacetoxy tetradecane	$C_{16}H_{29}F_3O_2$	310.39	None
11.011	4-Methyl (trimethylene) silyloxyoctane	$C_{12}H_{26}OSi$	214.42	None
11.095	Desulphosinigrin	$C_{10}H_{17}NO_6S$	279.31	Antiparasitic activity
12.191	1,25 dihydroxy vitamin D3	$C_{27}H_{44}O_3$	416.6	Bone density conservative agent, calcium channel agonist, antirachitic, antipsoriatic agent [26,27]
12.423	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.32	Anti-inflammatory, anticancer [26], antioxidant agent [34]
12.473	Butylated hydroxytoluene	$C_{15}H_{24}O$	220.35	Antioxidant activity [35]
13.332	1-Hexadecanol, 2-methyl-	$C_{17}H_{36}O$	256.56	None
13.892	4-(2,4,4-Trimethyl-cyclohexa-1,5-dienyl)-butyl	$C_{13}H_{18}O$	190.28	None
17.601	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194.18	Contrasting agent in tumor detection [36]
18.571	Phytol	$C_{20}H_{40}O$	296.5	Antioxidant, anticancer, and antitrypanosomal agent [37]
18.999	9,12,15-Octadecatrienoic acid	$C_{18}H_{30}O_2$	278.4	None
19.448	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan	$C_{28}H_{44}O_4$	444.6	None
19.760	Glycine, *N*-(*N*-glycyl-L-leucyl)-	$C_{10}H_{19}N_3O_4$	245.28	None
20.857	Methyl glycocholate	$C_{36}H_{69}NO_6Si_3$	696.2	None
23.057	Heptasiloxane, hexadecamethyl-	$C_{16}H_{48}O_6Si_7$	533.1	None
23.516	Oleic acid, 3-(octadecyloxy) propyl ester	$C_{39}H_{76}O_3$	593	Antifungal activity [38], antibacterial activity
24.747	Squalene	$C_{30}H_{50}$	410.7	Adjunctive agent in cancers, protective agent in chemotherapy [37]
24.935	Lupeol	$C_{30}H_{50}O$	426.7	Anticancer, anti-inflammatory [39], Antiapoptotic agent
28.077	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366.67	None
28.882	dl-a-Tocopherol	$C_{29}H_{50}O_2$	430.7	Used in the treatment of muscular dystrophy [40]

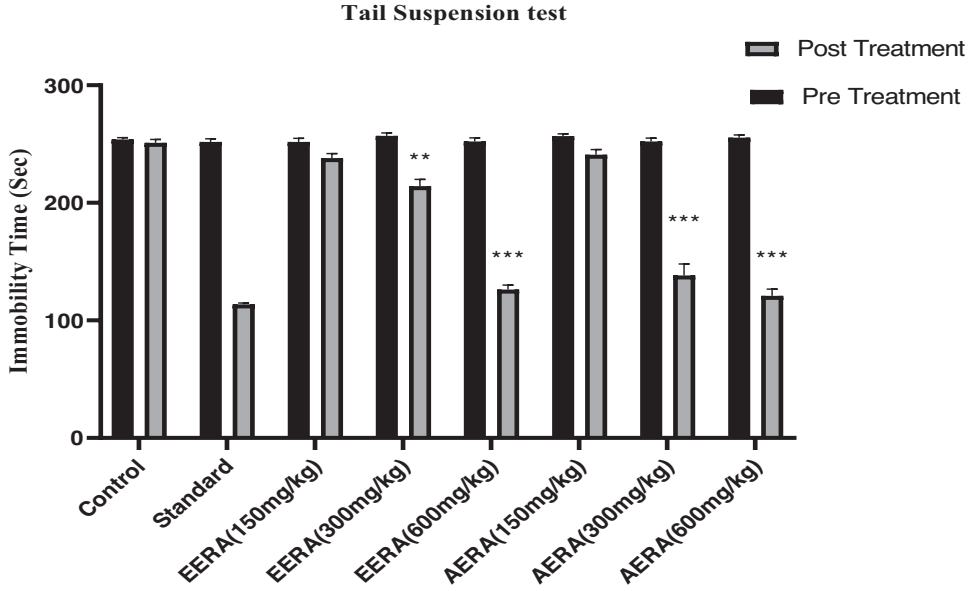
improving potency using the radial arm maze and Y-maze tests. In the radial arm maze test, EERA 300 and 600 mg/kg treatment significantly decreased reference memory error ($P<0.01$), spatial working memory error ($P<0.001$), and transfer latencies ($P<0.001$) compared with the scopolamine-treated rats. However, the AERA 200 and 400 mg/kg significantly reduced reference memory error with $P<0.001$, spatial working memory error ($P<0.001$), and transfer latency ($P< 0.0001$) in scopolamine-treated animals.

Figure 1



Results of the forced swim test. The graph shows immobility time (Sec), (n=6, Mean±SEM). The significance of the activity was indicated based on P values; P value was found not significant for 150 mg/kg of EERA and100 mg/kg of AERA; $P<0.01$(**) for 200 mg/kg of AERA; $P<0.001$ (***) for 300 and 600 mg/kg of EERA and 200 mg/kg AERA; $P<0.001$(***) when compared with the pretreatment groups.

Figure 2

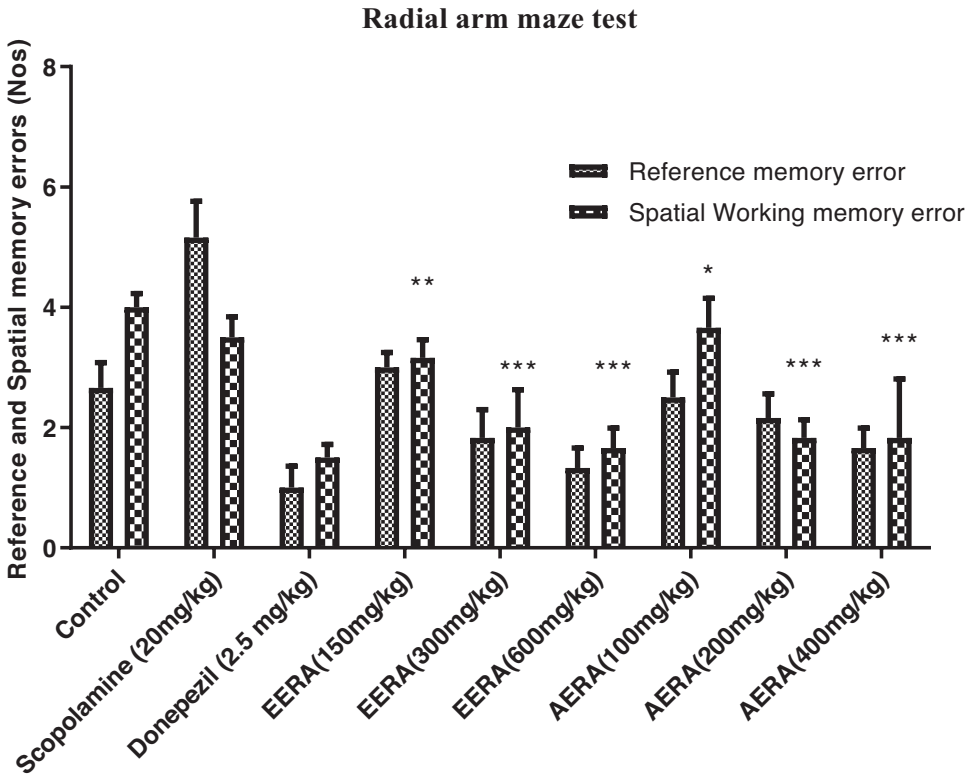


Results of the tail suspension test. The graph shows immobility time (n=6, Mean±SEM); the significance of the activity was indicated based on P values. Groups treated with EERA (150 mg/kg) and AERA (100 mg/kg) have not shown any significance; $P<0.001$ (**) for 300 mg/kg of EERA is significant and $P<0.001$(***) for EERA (600 mg/kg); 200 and 400 mg/kg of AERA is significant when compared with the pretreatment groups.

The % spontaneous alterations in treated and untreated animals are indicated in Fig. 5 at tested doses of both extracts. In the Y-maze test, the administration of AERA 400 mg/kg and EERA 600 mg/kg treatments exhibited significant increase in spontaneous alterations ($P<0.0001$).

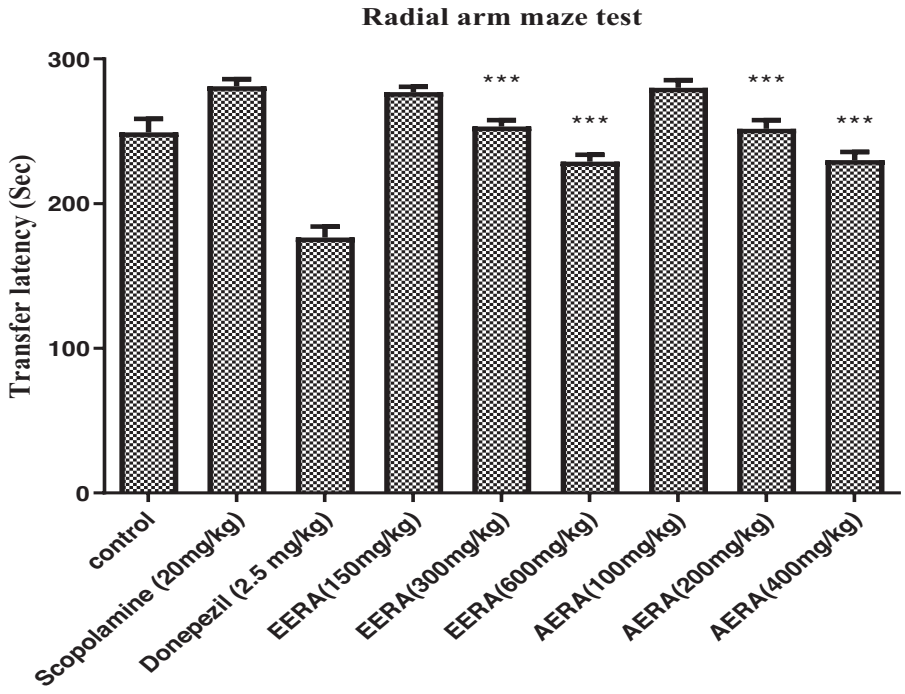
Natural resources and their derivatives, such as alkaloids and flavonoids, have proven to be reliable leads for the development of novel neuroprotective drugs that can be used to treat neurodegenerative diseases like Alzheimer's and Parkinson's diseases [41]. Numerous studies have investigated the antiaging effects of flavonoids, alkaloids, and phenolic chemicals on the brain. The plant extract has shown to have good potential in preserving the neuronal kappa/beta subunits as a result of the phytochemical screening demonstrating the

Figure 3



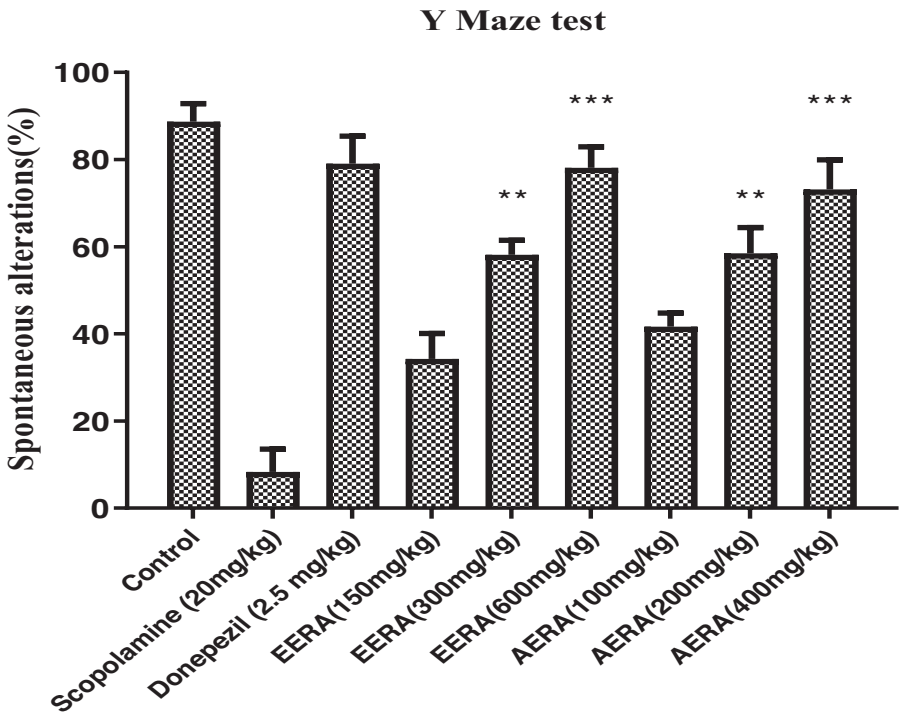
Results of the radial arm maze test. Graphs show reference memory error and spatial working memory errors (No's) (n=6, mean±SEM). The significance of the activity in terms of reference and special memory errors was indicated based on P values, $P<0.05$ (*) for 100 mg/kg of AERA; $P<0.01$ (**) for 150 mg/kg of EERA; $P<0.001$ (***) for 300 and 600 mg/kg of EERA; $P<0.001$(***) for 200 and 400 mg/kg of AERA, when compared with the pretreatment groups.

Figure 4



Results of the radial arm maze test. The graph shows transfer latency time (s) (n=6, Mean±SEM); the values with *** significant at $P<0.0001$ for 300 and 600 mg/kg of EERA; *** significant at $P<0.0001$ for 200 and 400 mg/kg of AERA when compared with the control group.

Figure 5



Results of the Y-maze test. The graph shows % spontaneous alterations (n=6, mean±SEM), the values with *** significant at $P<0.0001$ for 600 mg/kg of EERA and 400 mg/kg of AERA; ** significant at $P<0.001$ for 300 mg/kg of EARA and 200 mg/kg of AERA when compared with the control group.

occurrence of such constituents. The fundamental function of the neuronal kappa and beta receptors is the regulation of synaptic plasticity and memory [42]. *Ginkgo biloba* and other naturally occurring herbs with antioxidant activity have also demonstrated to have additional neuronal advantageous actions like anti-inflammatory [43], metal chelating, and acetylcholine esterase (AchE) inhibitory effects.

The chosen herb has been reported to have anti-inflammatory [44] and antioxidant [45] effects. In addition, the plant's leaves displayed signs of central analgesic activity in our earlier research [7]. According to earlier tests [7], the EERA extract did not exhibit any toxic indications even at higher doses (2000 mg/kg); henceforth, it was regarded as safe and the animals were given up to 600 mg/kg in the present study. However, as it was observed to have mild hepatotoxicity at 2000 mg/kg [7], the maximal dose of AERA extracts was taken up to 400 mg/kg. In the general context, antidepressant medications have also been demonstrated to reduce anxiety and improve memory [46]. To assess the antidepressant activity, TST [15] and FST [14] were used. Imipramine [47], a tricyclic antidepressant that was used as a standard, works by preventing the reuptake of noradrenaline and serotonin; as a result, the levels of these neurotransmitters in the brain are increased. The immobility period has been used as a criterion to assess the effects of antidepressants. The immobility period in the TST and FST has significantly decreased with increased doses of EERA and AERA.

To assess the nootropic action of extracts, a small approach was used because antidepressant medications have the effect of reducing neurotransmitter levels in the brain. Nootropic substances, also known as cognitive enhancers, have been used to treat neurodegenerative diseases and increase learning, memory, and attention, particularly in the elderly. Scopolamine is being used to impair the animals' cognitive abilities in tests like the passive avoidance test, radial arm maze, Y-maze, and T-maze to evaluate natural substances for their nootropic activity. The natural alkaloid scopolamine [47], which is derived from the plant *Datura stramonium*, could reduce the levels of Ach in the brain and impair short- and long-term memories in both human and animal models.

Since scopolamine is a model that can be used to justify the induction of cognitive impairment, it was taken into consideration. In this study, the radial arm maze and the Y-maze tests were used to assess the cognitive

boosting activities. Reference memory error, spatial working memory errors, and transfer latencies in the radial arm maze animal model were assessed [16], in which both EERA and AERA at medium and high doses considerably decreased these key variables. The decrease in the indicators may indicate that the plant extract has cognitive boosting activity. Detecting spontaneous altering errors in the Y-maze test is the criteria used to evaluate spatial short-term memory.

The test samples of EERA and AERA have suppressed spontaneous alteration errors in high doses in the Y-maze model. The administration of any of the extracts does not significantly affect the number of entries into the arms. In the general context, neurons consist of characteristics including excitability, which refers to a neuron's capacity to respond to stimuli, and conductivity, which refers to a neuron's capacity to transfer nerve impulses to other neurons, primarily through neurotransmitters. Besides neuronal loss, neurotransmitter levels are also suppressed in neurodegenerative illnesses in Alzheimer's and Parkinson's conditions. In animal models of scopolamine-induced neurodegeneration, *R. apiculata* leaf extracts improved cognition-enhancing capacity.

The phytochemicals comprised extracts, such as flavonoids, alkaloids, and/or phenolic compounds, may be implicated in this kind of medicinal property. Therefore, both extracts were subjected to an experimental test to determine the total flavonoid content using the $AlCl_3$ method assuming their significant role in imparting neurological benefits over other phytochemicals. Surprisingly, both extracts had a significant number of flavonoids, in which the EERA had a little more abundant of flavonoid composition than AERA. The GC-MS analysis of the plant extracts was proven to have therapeutically useful phytochemicals. The distribution of various bioactive compounds as per GC-MS reports could be the reason behind possessing nootropic activities of plants. To explore the effect of the plant constituents in altering the levels of brain neurotransmitters and the histology of the brain, further research is needed. Furthermore, to identify the functional groups that are responsible for pharmacological activity and to characterize them, further study is needed on processes such as phytochemical isolation.

Conclusion

The results of the current investigation unequivocally demonstrate that *R. apiculata* leaves have antidepressant and cognition-improving properties for EERA (at 300 mg/kg, 600 mg/kg) and AERA (at 200 mg/kg, 400 mg/kg). These findings lead us to the plant's effectiveness in the treatment and management of several neurological disorders. The findings of study prompted us to extend the investigations to estimate the levels of various neurotransmitters and biological amines, which may further disclose the beneficial effect of this plant in treating neurological illnesses.

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Conflicts of interest

There are no conflicts of interest.

References

1 Brigitta B. Pathophysiology of depression and mechanisms of treatment. Dialogues Clin Neurosci 2002; 4:7–20.

2 Froestl W, Muhs A, Pfeifer A. Cognitive enhancers (nootropics). Drugs interacting with receptors. J Alzheimer's Dis 2014; 41:961–1019. Update 2014

3 Malik J, Karan M, Vasisht K. Nootropic, anxiolytic and CNS-depressant studies on different plant sources of shankhpushpi. Pharm Biol 2011; 49:1234–42.

4 Friess DA. Mangrove forests. Curr Biol 2016; 26:R746–8.

5 Sachithanandam V, Lalitha P, Parthiban A, Mageswaran T, Manmadhan K, Sridhar R. A review on antidiabetic properties of Indian mangrove plants with reference to island ecosystem. Evid Based Complement Alternat Med 2019; 2019:1–21.

6 Saddhe AA, Malvankar MR, Kumar K. Selection of reference genes for quantitative real-time PCR analysis in halophytic plant *Rhizophora apiculata*. Peer J 2018; 6:e5226.

7 Mande A, Malothu N, Banadaru N, Murthy TE. Acute oral toxicity studies and evaluation of central analgesic activity of various extracts of leaves of *Rhizophora apiculata*. Toxicol Int 2022; 239–56

8 Selvaraj G, Kaliamurthi S, Thirugnasambandan R. Effect of glycosin alkaloid from R apiculata in non-insulin dependent diabetic rats and its mechanism of action: in vivo and in silico studies. Phytomedicine 2016; 23:632–40.

9 Martins J, Brijesh S. Phytochemistry and pharmacology of anti-depressant medicinal plants: A review. Biomed Pharmacother 2018; 104:343–365.

10 Li MX, He XR, Tao R, Cao X. Phytochemistry and pharmacology of the genus Pedicularis used in traditional Chinese medicine. Am J Chin Med 2014; 42:1071–98.

11 Khandelwal K. Practical pharmacognosy. Nirali Prakashan, Pune, India: Pragati Books Pvt. Ltd; 2008.

12 Mathur R, Vijayvergia R. Determination of total flavonoid and phenol content in Mimusops elengi Linn. Int J Pharm Sci Res 2017; 8:5282–5.

13 Guguloth SK, Malothu N, Dsnbk P. GC-MS analysis and thrombolytic property of methanolic leaf extracts of Terminalia pallida brandis against carrageenan instigated tail thrombosis model in mice. Pharmacogn Res 2022; 14:53–60.

14 Shoeb A, Chowta M, Pallempati G, Rai A, Singh A. Evaluation of antidepressant activity of vanillin in mice. Indian J Pharmacol 2013; 45:141–44.

15 Cryan JF, Mombereau C, Vassout A. The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. Neurosci Biobehav Rev 2005; 29:571–625.

16 Talpate KA, Bhosale UA, Zambare MR, Somani RS. Neuroprotective and nootropic activity of *Clitorea ternatea* Linn. (Fabaceae) leaves on diabetes induced cognitive decline in experimental animals. J Pharm Bioallied Sci 2014; 6:48–55.

17 Kailas KM, Sutar GV, Remeth JD, Devade OA. Evaluation of nootropic activity of *Limonia acidissima* against scopolamine-induced amnesia in rats. Turk J Pharm Sci 2021; 18:3–9.

18 Xin L, Che B, Zhai B, Luo Q, Zhang C, Wang J, *et al.* 1, 25–Dihydroxy vitamin D3 attenuates the oxidative stress–mediated inflammation induced by P M2. 5via the p38/NF-κB/NLRP3 pathway. J Inflamm 2019; 42:702–13.

19 Saeb S, Azari H, Mostafavi-Pour Z, Ghanbari A, Sepideh Ebrahimi M, Mokarram P. 9-cis-retinoic acid and 1, 25–dihydroxy vitamin D3 improve the differentiation of neural stem cells into oligodendrocytes through the inhibition of the Notch and Wnt signaling pathways. Iran J Med Sci 2018; 43:523–32.

20 Parang K, Knaus EE, Wiebe LI, Sardari S, Daneshtalab M, Csizmadia F. Synthesis and antifungal activities of myristic acid analogs. Arch Pharm 1996; 329:475–82.

21 Villanueva GR, Monte MJ, Barbero ER, Serrano MA, Marin JJ. Evidence for dual effect of bile acids on thymidine anabolism and catabolism by the regenerating rat liver. Biochim Biophys Acta 1996; 1289:136–44.

22 Thakur RS, Ahirwar B. A steroidal derivative from Trigonella foenum graecum L. that induces apoptosis *in vitro* and *in vivo*. J Food Drug Anal 2019; 27:231–9.

23 Johnson TO, Odoh KD, Nwonuma CO, Akinsanmi AO, Adegboyega AE. Biochemical evaluation and molecular docking assessment of the anti-inflammatory potential of Phyllanthus nivosus leaf against ulcerative colitis. Heliyon 2020; 6:e03893.

24 Moni SS, Alam MF, Sultan MH, Makeen HA, Alhazmi HA, Mohan S, *et al.* Spectral analysis, in vitro cytotoxicity, and antibacterial studies of bioactive principles from the leaves of Conocarpus lancifolius, a common tree of Jazan, Saudi Arabia. Braz J Biol 2021; 83:e244479

25 Udaya Prakash NK, Sripriya NS, Raj DD, Deepa S, Bhuvaneswari S. Antioxidant potency and GC-MS composition of Origanum majorana Linn. Pak J Pharm Sci 2019; 32:2117–2122.

26 Huang L, Su T, Li X. Natural products as sources of new lead compounds for the treatment of Alzheimer's disease. Curr Top Med Chem 2013; 13:1864–78.

27 Hardman JG, Limbird LE, Gilman AG. *Goodman* and Gilman's The Pharmacological Basis of Therapeutics. 10th ed. New York, NY: McGraw-Hill, 2001; 1729.

28 McEvoy GK, (ed). American Hospital Formulary Service- Drug Information 2005. Bethesda, MD: American Society of Health-System Pharmacists, Inc; 2005. (Plus Supplements), 3542

29 Keshav P, Goyal DK, Kaur S. GC-MS screening and antiparasitic action of Putranjiva roxburghii leaves against sensitive and resistant strains of Leishmania donovani. J Parasit Dis 2021; 45:1002–13.

30 Sehgal AA, Li Y, Lal B, Yadav NN, Xu X, Xu J, *et al.* CEST MRI of 3-O-methyl-D-glucose uptake and accumulation in brain tumors. Magn Reson Med 2019; 81:1993–2000.

31 Nag A, Verma P, Paul S, Kundu R. *In silico* analysis of the apoptotic and HPV inhibitory roles of some selected phytochemicals detected from the rhizomes of greater cardamom. Appl Biochem Biotechnol 2022; 10:1–25.

32 Poochi SP, Easwaran M, Balasubramanian B, Anbuselvam M, Meyyazhagan A, Park S, *et al.* Employing bioactive compounds derived from Ipomoea obscura (L.) to evaluate potential inhibitor for SARS-CoV-2 main protease and ACE2 protein. Food Front 2020; 1:168–79.

33 Reddy LH, Couvreur P. Squalene: A natural triterpene for use in disease management and therapy. Adv Drug Deliv Rev 2009; 61:1412–26.

34 Marchese A, Orhan IE, Daglia M, Barbieri R, Di Lorenzo A, Nabavi SF, *et al.* Antibacterial and antifungal activities of thymol: A brief review of the literature. Food Chem 2016; 210:402–14.

35 Nair RV, Jayasree DV, Biju PG, Baby S. Anti-inflammatory and anticancer activities of erythrodiol-3-acetate and 2, 4–di–tert–butylphenol isolated from Humboldtia unijuga. Nat Prod Res 2020; 34:2319–22.

36 Choi SJ, Kim JK, Kim HK, Harris K, Kim CJ, Park GG, *et al.* 4-Di-tert-butylphenol from sweet potato protects against oxidative stress in PC12 cells and in mice. J Med Food 2013; 16:977–83.

37 Saad SB, Ibrahim MA, Jatau ID, Shuaibu MN. The therapeutic potential of phytol towards Trypanosoma congolense infection and the inhibitory effects against trypanosomal sialidase. Exp Parasitol 2020; 216:107943.

38 Abubacker MN, Devi PK. *In vitro* antifungal potentials of bioactive compound oleic acid, 3-(octadecyloxy) propyl ester isolated from *Lepidagathis cristata* Willd. (Acanthaceae) inflorescence. Asian Pac J Trop Med 2014; 7:S190–3.

39 Liu K, Zhang X, Xie L, Deng M, Chen H, Song J, *et al.* Lupeol and its derivatives as anticancer and anti-inflammatory agents: Molecular mechanisms and therapeutic efficacy. Pharmacol Res 2021; 164:105373.

40 Hafner PG, Anderson R. Treatment of progressive muscular dystrophy with synthetic dl-a-tocopherol. Northwest Med 1946; 45:256–8.

41 Prabhu V, Guruvayoorappan C. Protective effect of marine mangrove *R. apiculata* on acetic acid induced experimental colitis by regulating anti-oxidant enzymes, inflammatory mediators and nuclear factor-kappa B subunits. Int Immunopharmacol 2014; 18:124–34.

42 Della Loggia R, Sosa S, Tubaro A, Bombardelli E. Anti-inflammatory activity of Ginkgo biloba flavonoids. Planta Med 1993; 59:A588.

43 Gao M, Xiao H. Activity-guided isolation of antioxidant compounds from Rhizophora apiculata. Molecules 2012; 17:10675–82.

44 Loo AY, Jain K, Darah I. Antioxidant and radical scavenging activities of the pyroligneous acid from a mangrove plant, Rhizophora apiculata. Food Chem 2007; 104:300–7.

45 Rahmati B, Kiasalari Z, Roghani M, Khalili M, Ansari F. Antidepressant, and anxiolytic activity of *Lavandula officinalis* aerial parts hydroalcoholic extract in scopolamine-treated rats. Pharm Biol 2017; 55:958–65.

46 Froestl W, Muhs A, Pfeifer A. Cognitive enhancers (nootropics). Part 1: drugs interacting with receptors. J Alzheimer's Dis 2012; 32:793–887.

47 Kouémou NE, Taiwe GS, Moto FC, Pale S, Ngoupaye GT, Njapdounke JS, *et al.* Nootropic and neuroprotective effects of Dichrocephala integrifolia on scopolamine mouse model of Alzheimer's disease. Front Pharmacol 2017; 8:847.