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Exploring the antidepressant and anxiolytic effects of *Desmostachya bipinnata*: Evidence from animal models and *in-silico* studies

Muhammad Muzammil Nazir[1], Muhammad Arif[2], Mahnoor Arif[3], Iqra Farzeen[1], Anjum Khursheed[4], Asma Ashraf[1*], Hareem Noor[1], Muhammad Haneef[5], Abdulhakeem S Alamri[6,7], Majid Alhomrani[6,7], Walaa F Alsanie[6,7] and Hammad Saleem[8*]

[1]Department of Zoology, Government College University, Faisalabad, Pakistan
[2]Bolan Medical College Hospital, Quetta, Pakistan
[3]Tertiary Care Hospital, Nishtar-II, Multan, Pakistan
[4]Faculty of Pharmacy, Grand Asian University, Sialkot, Pakistan
[5]Department of Pharmacy, Benazir Bhutto Shaheed University Lyari, Karachi, Pakistan
[6]Department of Clinical Laboratory Sciences, The Faculty of Applied Medical Sciences, Taif University, Taif, Saudi Arabia.
[7]Research Centre for Health Sciences, Taif University, Taif, Saudi Arabia
[8]Institute of Pharmaceutical Sciences (IPS), University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan

Abstract: The purpose of this study was to assess the ethanolic extract of *Desmostachya bipinnata* (EEDBR)'s sedative and antidepressant properties in Swiss albino mice. The extract was given to Swiss albino mice in single doses of 100, 200 and 400mg/kg of body weight for different biological tests. In the open field test, the diazepam group and the 400mg/kg dosage group spent more time in the centre zone than the control group. The 400mg/kg dosage and diazepam group had no discernible effect on centre time response. Additionally, a dosage of 100mg/kg exhibits a significant impact of 44±3.60. Compared to treated with EEDBR, those given with standard control Diazepam (1mg/kg) had higher head dips. At all dosages, there was a substantial decrease in locomotor activity in HCT as compared to the control group at all time intervals (between 30 and 120 minutes). Diazepam, the positive control, significantly lowers locomotor activity. EEDBR reduced sadness and anxiety in a dose-dependent way. Higher dosages of EEDBR result in noticeably stronger antidepressant effects, indicating a dose-dependent connection. For the molecules isopulegone, geranyl isovalerate and eucalyptol, the *in-silico* docking scores for Cyclooxygenase-1 and Cyclooxygenase-2 are -8.2, -6.2, -8.4 and -8.9, -7.2, -7.6 respectively.

Keywords: Antidepressant, *Desmostachya bipinnata,* anxiety, sedative, *in-silico,* docking.

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INTRODUCTION

Depression is defined as the presence of depressive symptoms, psychological distress, or mental distress. A combination of hereditary and environmental factors contributes to depression (Kong *et al*., 2024; Zajkowska *et al*., 2021). Depression can also manifest physically, with sufferers experiencing feelings of sadness or tears, exhaustion, guilt, agitation or rage, low self-esteem and disappointment or hopelessness. Currently, 7.3% of people worldwide suffer from anxiety, making it one of the most common mental disorders. This illness is characterized by alarming threats and complex feelings of bewilderment and discomfort. There are many neurotransmitters in the central excitatory (glutamatergic) and inhibitory (GABAergic) inputs balance to control the nervous system (CNS) and neuronal activity (Kang *et al*., 2018; Li *et al*., 2022). In the world, anxiety and depression are thought to be the two most prevalent neurological conditions. Anxiety and sleeplessness can arise from even a little reduction in GABAergic activity. Benzodiazepines play a major role in treating anxiety and work by increasing GABAergic inhibition; nonetheless, they have the potential to be abused and have potentially fatal side effects (Wang *et al*., 2022). Although the dosages needed to treat anxiety are higher than those needed to treat depression, selective serotonin reuptake inhibitors, or SSRIs, are becoming more and more common. Furthermore, using them may increase the risk of suicide, deteriorate behavior and cause withdrawal symptoms (Heissel *et al*., 2023).

Depression affects around 300 million individuals globally, with a 4.2% prevalence. It is estimated that neurological conditions impact 450 million individuals worldwide and depression and anxiety affect over 0.12 billion people (Goni *et al*., 2021). One depressive episode is said to affect about 1 in 5 persons, with women two times more likely than men to suffer one. Hippocampal atrophy, impaired neural plasticity with diminished neurogenesis, dysregulation of brain circuits, deficits in monoamine neurotransmitters and lower levels of glial fibrillary acidic protein in the prefrontal cortex of the brain are the main contributing causes (Feng *et al*., 2023;

**Corresponding authors:* e-mail: asmabinm@gmail.com; hammad.saleem@uvas.edu.pk

Kozubski *et al*., 2021). Patients' risk of suicide has drastically decreased with the development of antidepressant medications. Limitations such as poor tolerability, high side effects and limited remission provide strong reasons to search for an alternate medication to help depressed individuals. According to recent surveys, 80% of the population in developing nations still relies on herbal therapies because of their cost-effectiveness and safe results, which have drawn people back to nature (Ormel *et al*., 2022).

It is noted that monoamine oxidase inhibitors and tricyclic antidepressants decreased while those for other classes increased. Tricyclics, monoamine oxidase inhibitors like mirtazapine are example of manufactured antidepressant medications (Pricaud *et al*., 2022). Although antidepressant drugs reduce aberrant brain activity, they may have unfavorable side effects (Ormel *et al*., 2022).

D. bipinnata commonly known as dub found in grassy areas of Pakistan. Native to Asia and Africa, *D. bipinnata* is a perennial grass species also referred to as Indian doab or alfa grass. *(Vivekanandarajah & Rajamanoharan, 2021)*. *D. bipinnata* used as a pasture crop in Pakistan and is essential for reclaiming saline desert soils and uncultivated areas (Shaheen *et al*., 2020).

Pharmacological uses of *D. bipinnata* include antioxidant properties, antibacterial activities (Alqudah *et al*., 2023), anti-inflammatory (Jaouani *et al*., 2024)(Ndhlala *et al*., 2024), antifungal (Hamida *et al*., 2024) and immunomodulatory effects (Batiha *et al*., 2023). *D. bipinnata* is used in the past to treat urinary tract stones (Ammor *et al*., 2020). The pharmacological activities of *D. bipinnata* are analgesic activity, anti-diabetic activity, anti-histaminic activity, antimicrobial (Shrestha *et al*., 2021), anti-hepatotoxicity, antibiotic activity and cytotoxic activity. *D. bipinnata* is traditionally used to treat relaxation and stress relief, respiratory health, skin care, wound healing, digestive comfort, insect repellent and traditional crafts (Putta *et al*., 2023). Populations of *D. bipinnata* exhibited significant diversity, giving this species a great chance to live in dry, arid environments influenced by salt (Bibi *et al*., 2024). Given its historical use in treating anxiety, sleeplessness, hysteria, skin irritation, coughing, and fever, the roots of *D. bipinnata* were chosen for testing their anxiolytic and antidepressant properties. However, no scientific study of this species' antidepressant effect in experimental animal models has been published in the literature. Thus, the current investigation examined the sedative and anxiolytic effects of EEDBR on Swiss albino mice.

MATERIALS AND METHODS

Plant collection

Desmostachya bipinnata roots were selected for the comparative study of antidepressants activity in Swiss albino mice. The collected roots were authenticated by a botanist of the Department of Botany, Government College University Faisalabad (GCUF).

Preparation of extract

The dried parts of roots of *D. bipinnata* were powdered (250g) and macerated in 500ml of 99.99% pure ethanol. 250g of powdered plants were used for each extraction by using Soxhlet extraction for about 72 hours. The rotary evaporator (R-215 Labor technik AG, Flawil, Japan) was used to concentrate the extract obtained under reducing the pressure and temperature (65◦C). It is used to separates the ethanol and concentrated plant extract is collected. The percentage yield was 9% (Wang & Peng, 2023).

Ethical clearance

The Institutional Animal Ethics Committee (IACE) has granted its ethical clearance. The Institutional Animal Ethical Committee of GC University in Faisalabad accepted the protocol utilised in this work to use mice as an animal model for research on antidepressants (NIMSUR/IAEC/CERT/2014/07/04). Attached is a copy of the Institutional Animal Ethical Committee (IAEC) ethical clearance certificate.

Animals

Swiss albino mice were used. Swiss albino mice measuring 32-36g were kept in polycarbonate cages that measured 30cmx20cmx13cm under standard laboratory conditions. The experimental conditions included maintaining a temperature of 25°C, humidity levels between 55% and 56% and a consistent daily light cycle of 12 hours. Mice were acclimatized for 14 days before start of experiments. We used 5 groups with a repetition of 3 rats per group, resulting in a total of 75.

Experimental protocols

Each of the five groups of animals was randomly assigned to either the experimental or control group. Normal saline was given to the animals in group 1. Diazepam was given to the animals in group 2. *D. bipinnata* extracts were given to animals in groups 3, 4 and 5 at dosages of 100, 200 and 400mg/kg.

Behavioral tests

Open field test (OFT)

There were thirteen squares (10 cm × 10 cm) cut out of the arena floor. The duration spent in the centre of the arena, the number of raising and grooming sessions, and the number of lines crossed were all recorded during the five (5) minutes of free imploration that each animal underwent one hour after treatment (Kouémou *et al*., 2024).

Elevated plus maze test (EPM)

The raised plus maze apparatus was made up of two 35 cm by 5 cm open arms and two 30 cm by 5 cm by 15 cm

closed arms that were all attached to a single 5 cm by 5 cm centre platform. The closed arms featured hardwood walls and black-painted floors. Mice weighing 35 to 40 grammes were used in the experiment. The mice were filmed for six minutes using a video camera. The mice were allowed to acclimatize to their environment for the first minute and for the next five minutes, the amount of time spent in the open arms and the quantity of admissions were noted (Uddin *et al*., 2018). According to the research design, experimental animals in each of the three groups control, standard, and test were dosed with the appropriate solutions (Reza *et al*., 2023).

Hole board test (HBT)

The hole board used in our experiment was 20 cm by 40 cm and included 16 uniformly spaced, 3 cm-diameter holes. The board hung 15 cm above the base and had a thickness of 1.8 cm. Five distinct groups of mice were created. The normal group had their therapy 15 minutes before the trial began, while the control and treatment groups received their treatments 30 minutes beforehand. A video camera was used to capture the exploratory behavior of each mouse for six minutes, with the last five minutes being the focus of data gathering. Each mouse was positioned separately in the middle of the board. The surrounding region was kept quiet to reduce disruptions. When the mouse's two eyes were seen going through a hole, a head dip was noted and the amount of time spent gazing through the hole was also timed (Reza *et al*., 2023).

Thiopental sodium-induced sleeping time test

Individual mice were given an injection of thiopental sodium (40mg/kg b. w., i. p.) half an hour after the treatment medication was given to induce sleep. Careful monitoring was used to record the length of sleep and the time after the thiopental sodium injection before the righting reflex was lost. After the animals were put in a dorsal decubitus position and put to sleep, the effects were observed. It was noted how long it took for the righting reflex, a sign of when sleep begins, to return after it had been lost (sleep time).

The ability of the animal to return to its typical position three times in a succession, signifying the restoration of the righting reflex, was considered recovery. This method provided valuable insights into the sedative properties of the chemical in experimental settings by enabling accurate evaluation and comparison of the sedative effects generated by different chemical concentrations (Ali *et al*., 2015).

Hole cross test (HCT)

A central partition separates a Hole Cross apparatus (30 × 20 × 14cm) into two equal sections, each measuring 15 × 10 × 14cm. The divider has a 3 cm hole in it that is 7 cm high (Nawrin *et al*., 2015). The mice were given free passage through the opening between compartments every three minutes and the number of crossings was noted (Khatoon *et al*., 2014).

In silico study

Swiss ADME analysis

D. bipinnata contain high concentration of phytocompounds such as flavonoids, carotenoids, alkaloids, phenols, tannins and terpenoids. We investigated the literature for bioactive chemicals and discovered active molecules in this plant. The in-silico research and ADME predictions for various drugs using the Swiss ADME online program (table 1). A 2D structural model, was generated in The SDF file which was shown in SMILES. Based on these features. The compounds selected for the initial screening were evaluated for their medicinal potential using Lipinski's Five Rule. The pharmacokinetics and therapeutic characteristics of the medicines were also studied.

Ligand preparation

The 2D structures from *D. bipinnata* phytocompounds are available on the PubChem website. Furthermore, ligand reduction and optimization were carried out with chem 3D pro and Chemdraw ultra-12.0.

Receptor preparation

The Protein Databank (PDB) provided the highest resolution protein X-ray structures, which were subsequently prepared for molecular docking studies using Maestro's Protein Preparation Wizard. This module processes the protein by adding hydrogen atoms, removing solvent, assigning bond ordering, generating disulfide bonds, filling in missing side chains and loops, and attaining a protonation level of 7.4 in the cell.

Docking simulation

Docking analysis was performed using PYRX (Farzeen *et al*., 2024). The ligand molecules' starting positions, orientations and torsions were chosen at random. A pool of 10 distinct samples was selected for each docking experiment and each cycle could only include 1.5A evaluations. Cyclooxygenase-1 (PDB code: 2OYE) and cyclooxygenase-2 (1CX2) were sourced from the Protein Data Bank. Docking calculations were performed using PYRX and BIOVIA Discovery Studio (http://www.3dsbiovia.com). Each docking experiment had ten consecutive runs, each with a maximum of 1.5 assessments.

STATISTICAL ANALYSIS

A statistical test of significance was considered statistically significant when $p<0.05$ was used (GraphPad Prism Version 6 for Windows, GraphPad Software)(Nazir *et al*., 2024). All the data are presented in mean values with standard deviation of mean. Mice in each were used in this experiment and one-way and two-way ANOVA to compare the groups. The mean ± standard deviation of the mean (SDM) is reported for each group. A P-value 0.05

was used to determine significant differences between means at the 95% confidence level.

RESULTS

This study analyzed sedative effect ethanolic extract from *D. bipinnata*. This scientific research has demonstrated the medicinal potential of *D. bipinnata* showing its pharmacological characteristics.

Open field test (OFT)

The OPF was used to evaluate anti-anxiety test of *D. bipinnata* roots ethanolic extract. Animals move towards corner when exposed to open areas as evidenced by the behavior of mice in the control group by time spent in crosses, line crossing, grooming, and rearing. However significant differences were observed in time by ANOVA as described in fig. 1. Statistical analysis showed a significant difference in line crossings between the control (73.33±4.1) and diazepam-treated group (52.33±2.51). Positive control (Diazepam) significantly reduced line crossing compared to vehicle control.

EEDBR treatment groups also showed a statistically significant effect in a dependent manner. 400mg/kg has significantly reduced time for line crossing (43±3.0) Grooming and rearing response was also decreased in IFT by increasing EEDBR. The grooming (22±1) and rearing response (36.33 ±1.52) were also decreased in the diazepam group as compared to the control 32.33±2.51 and 52.6±2.51 respectively. However prolonged time was spent in the center zone in the diazepam group (21.33±1.52) and at 400mg/kg dose (21±1.00) as compared to the control (31.33±1.50). In center time response, 400mg/kg dose and diazepam group has a nonsignificant effect.

Elevated plus maze test (EPM)

As presented in fig. 2, EEDBR at different doses significantly increased time spent in open arms as well as standard control (Diazepam) and negative control. EEDBR increased time spent in arms at all doses. Values are statistically significant as compared to control group "π" showed significant relative to standard group. Control: group 1% tween 80 in water (10mgl/kg p.o); SD: Diazepam 1mg/kg (i. p); 100, 200 and 400mg/kg treatment groups of EEDBR.

Highest stay time was observed (87.6±2.51) at 400mg/kg as well as in Diazepam group (101±1). Standard drug treated mice has more effect as compared to plant extract. EEDBR 400mg/kg increased response time (87.6±2.51) significantly as compared to control group (32±2.64).

Hole board test (HBT)

According to fig. 2, "α" shows a statistically significant effect as compared to the control group. "β" show significant result with the standard group (Diazepam) Control: group 1% tween 80 in water (10mgl/kg p.o); SD: Diazepam 1mg/kg (i. p); 100, 200 and 400mg/kg treatment groups of EEDBR. EEDBR showed anxiolytic effect at 100, 200 and 400mg/kg doses as compared to the negative control (32.66±2.51). High head dips show anxiety reduction. At 200 and 400mg/kg dose, head dips increased upto 50±2 and 62.66±2.5 respectively as compared to control (32.66±2.51). 100mg/kg dose has also a significant effect of 44± 3.60. Standard control Diazepam (1mg/kg) showed more head dips (52.6±2.51) as compared to EEDBR-treated groups.

Thiopental sodium-induced sleeping time test

According to fig. 2, it was found that onset time and sleep duration in thiopental induced sleep time test increased significantly in dose dependent manner. "ϖθ" shows statistically significant response in sleeping duration and "ωη" shows latency onset as compared to the standard and control group. Control: 1% tween 80 in water (10mgl/kg p.o); SD: Diazepam 1mg/kg (i.p); 100, 200 and 400mg/kg treatment groups of EEDBR. At 200 and 400mg/kg dose latency time was 9.3±2.08 and 14.6±1.52 while sleeping duration increased 51.66±22.51 and 75±23.0 respectively. In control group latency time was 49.3±2.51 and mice slept for 63.33±251. The latency onset (18±1) and sleeping duration (70±1.0) in Diazepam group was more significant as compared to EEDBR treatment groups.

Hole cross test (HCT

Most commonly used method for locomotor activity is OFT and HCT. As in the fig. 2, "α" shows statistically crossed holes as compared to control group. Control: 1% tween 80 in water (10mgl/kg p.o); SD: Diazepam 1mg/kg (i.p); 100, 200 and 400mg/kg treatment groups of EEDBR. The ethanolic extract showed statistically significant reduction in locomotor activity at all doses 100, 200 and 400mg/kg as compared to control group from at all time interval from 30 to 120 minutes. Positive control (Diazepam) has significant reduction in locomotor activity.

In silico study

Physiochemical properties

A medicine's physicochemical qualities are affected by its metabolism in the body. All chemicals satisfied Lipkin's criterion. Each compound has less than 10 rotatable bonds. Furthermore, each compound's molar ratio was within the permitted range (40-130). Topological polar surface area (TPSA) is a critical element in determining drug bioavailability. To maintain appropriate oral circulation, the water content of drugs should be evaluated using a Log-S value ranging from 1 to 8 (table 1).

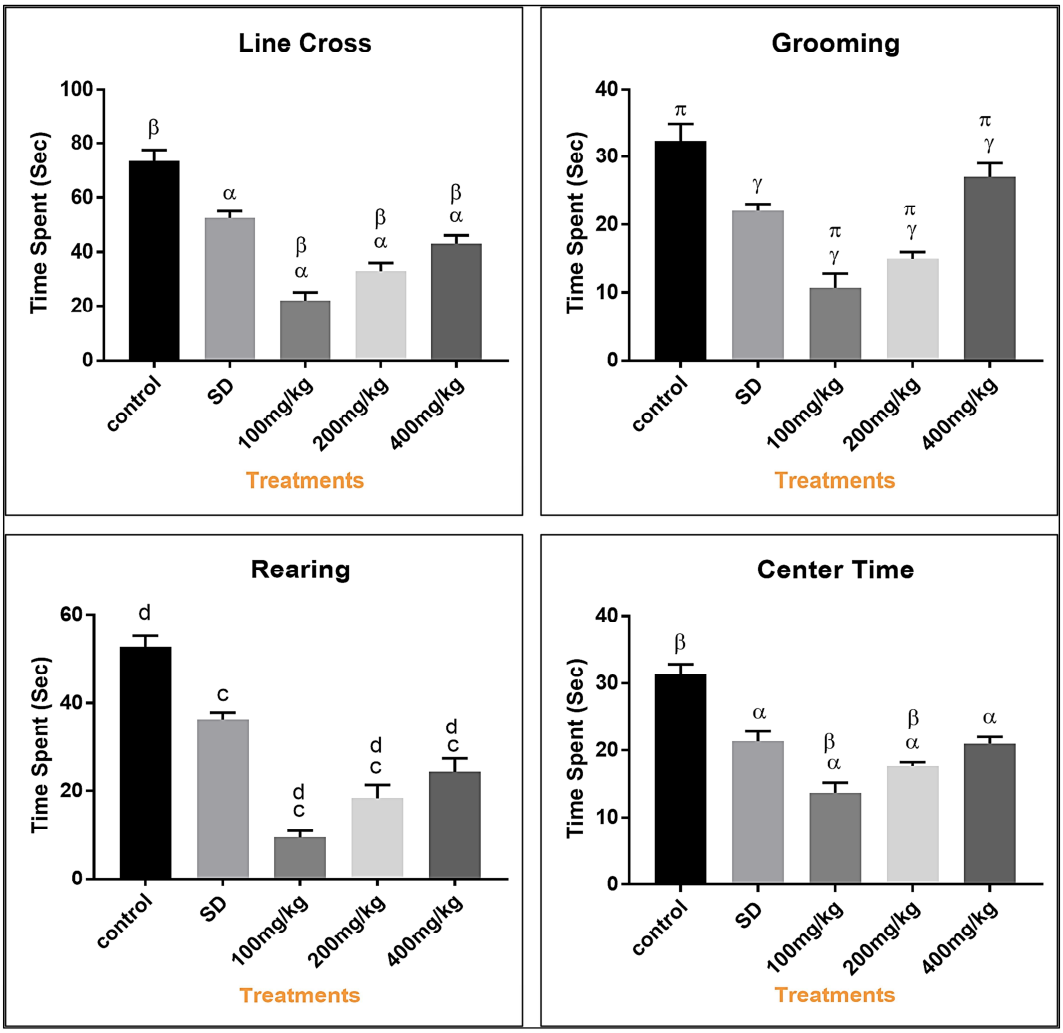

Fig. 1: EEDBR's effects on the centre time (A), grooming (B), rearing (C) and line crossings (D) of diazepam-treated mice in an open field. The values are shown as Mean± SD. When compared to the control group, values are deemed statistically significant ($p<0.05$). EEDBR treatment groups of 100, 200 and 400mg/kg; control: group 1% tween 80 in water (10 mgl/kg p.o.); SD: Diazepam 1 mg/kg (i.p.). Each symbol indicates statistical significance levels or comparisons between groups.

Table 1: Physicochemical and pharmacokinetic properties of EEDBR.

Phytochemical properties									
Compound	Formula	MW (g/mol)	Fraction Csp3	RB	HBA	HBD	MR	TPSA (Å²)	Log p
Isopulegone	$C_{10}H_{16}O$	152.23	0.70	1	1	0	47.80	17.07	2.25
Geranyl isovalerate	$C_{15}H_{26}O_2$	238.37	0.67	8	2	0	74.56	26.30	4.28
Eucalyptol	$C_{10}H_{18}O$	154.25	1.00	0	1	0	47.12	9.23	2.67
Pharmacokinetic properties									
Compound	GIA	BBB	P-gps	CYP1A2	CYPC19	Inhibitor CYP2C9	CYP2D6	CYP3A4	Log Kp (cm/s)
Isopulegone	High	Yes	No	No	No	No	No	No	-5.21
Geranyl isovalerate	High	Yes	No	Yes	No	Yes	No	No	-3.99
Eucalyptol	Low	Yes	No	No	No	Yes	No	No	-4.02

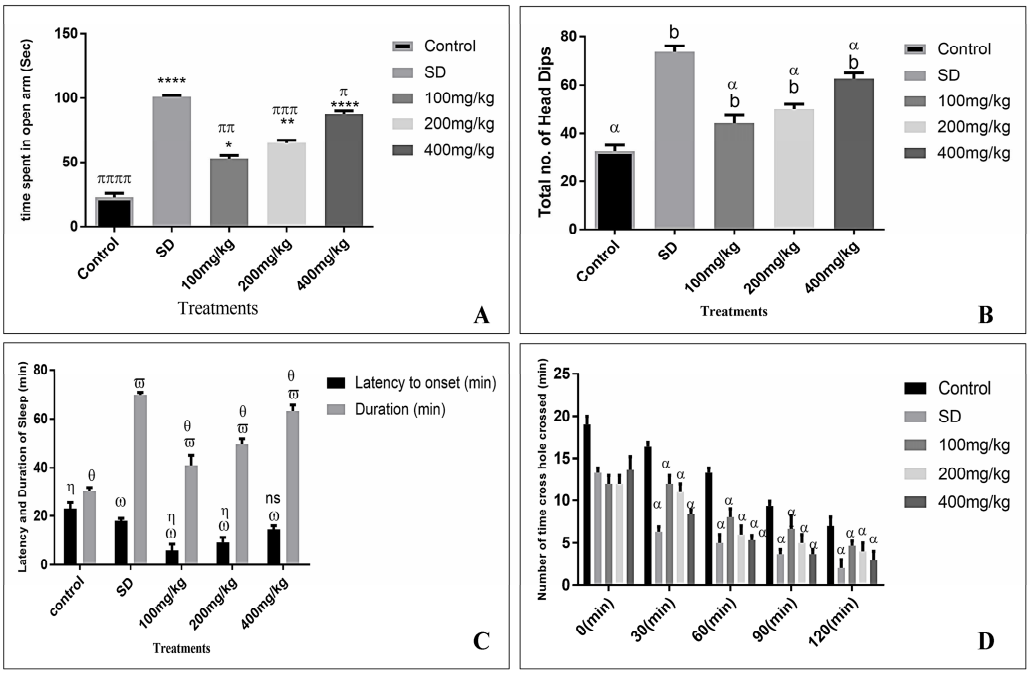

Fig. 2: (A) Effects of EEDBR on time spent in open arms in elevated plus maze test at p<0.05;(B) Effects of EEDBR on total number of head dips in hole board test at p<0.05;(C) Effects of *Desmostachyya bipinnata* ethanolic extract on thiopental-Na induced latent period on mice. Results are significant at p<0.05; (D) Effects of EEDBR extracts and diazepam on hole cross test. Results are significant at p<0.05. Values are presented as Mean± SD.

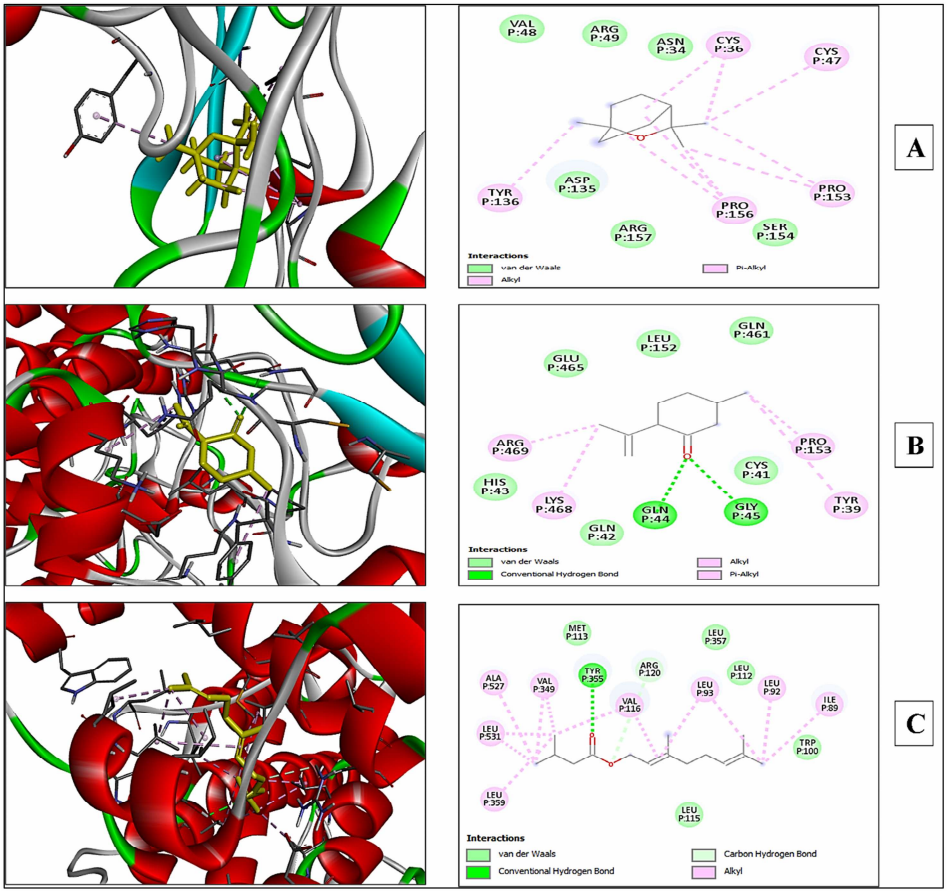

Fig. 3: (A) 2D and 3D view of Eucalptol with protein 2OYE, docking score (DS) -8.4; (B) 2D and 3D view of Isopulegone with protein 2OYE, DS -8.2; (C) 2D and 3D view of Geranayl isovalerate with protein 2OYE, DS -6.2.

Table 2: Lipophilicity and Drug-Likeness of EEDBR and toxicity of compounds through ADMET

	Isopulegone	Geranyl isovalerate	Eucalyptol
	Lipophilicity and Drug-Likeness		
Lipinski	Yes	Yes	Yes
Ghose	No	Yes	Yes
Veber	Yes	Yes	Yes
Egen	Yes	Yes	Yes
Muegge	No	Yes	Yes
BAS	0.55	0.55	0.55
PAINS	0	0	0
Lead Likeness	No	No	No
SA	2.75	3.04	3.65
Likeness	0.29	0.29	0.29
	Toxicity Table		
AMES toxicity	Non-AMES toxic	Non-AMES toxic	Non-AMES toxic
Carcinogens	Carcinogens	Carcinogens	Non-carcinogens
Acute oral toxicity	III	III	III
Rat acute toxicity	-2.1174	1.6174	1.8144

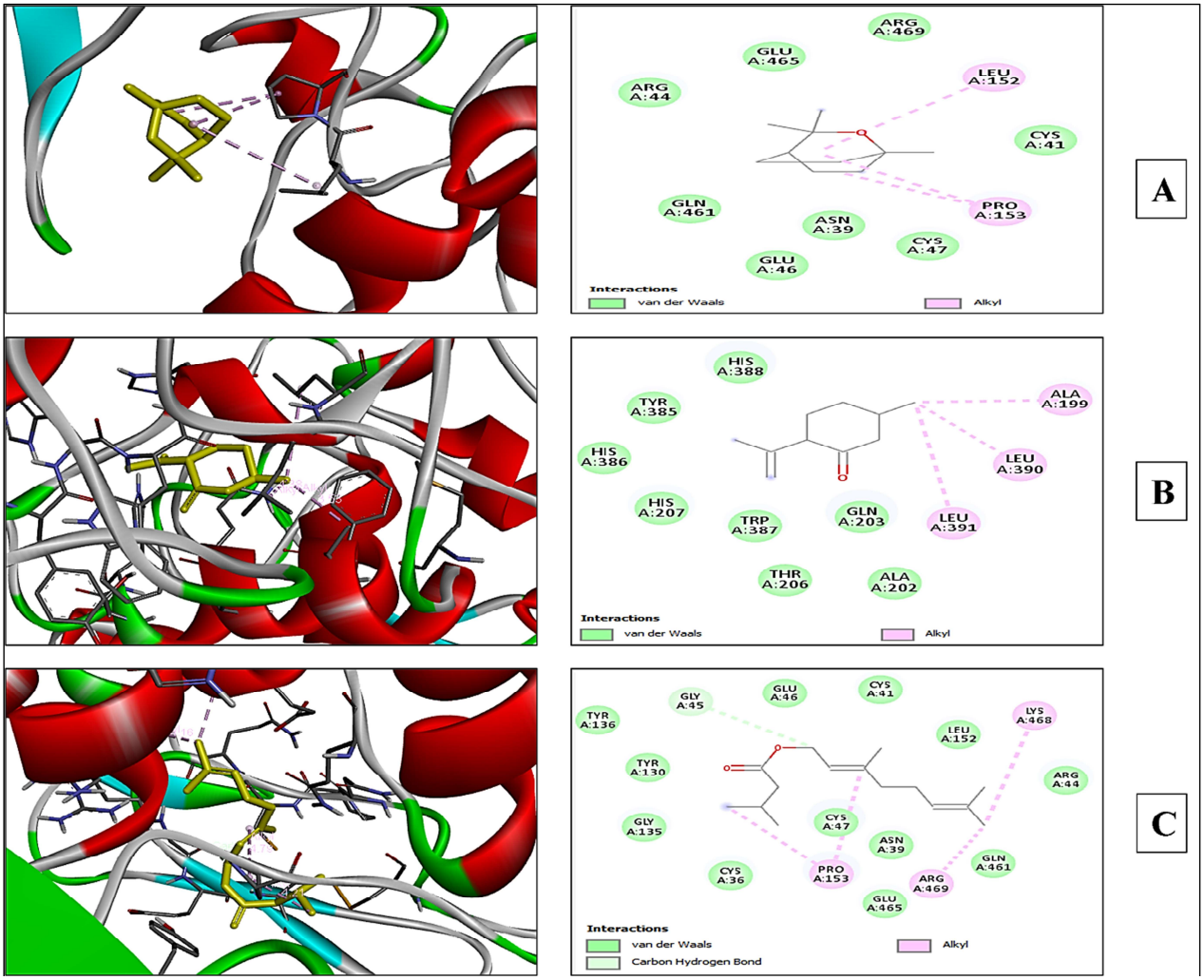

Fig. 4: (A) 2D and 3D view of Eucalptol with protein1CX2, docking score (DS) -7.6; (B) 2D and 3D view of Isopulegone with protein1CX2, DS -8.9; (C) 2D and 3D view of Geranayl isovalerate with protein1CX2, DS -7.2.

Pharmacokinetics properties

The blood-brain barrier (BBB) represents the interaction of blood and brain tissue drugs. P-gps must be inhibited in order for phospholipoids and the phenyl ring to form. The cytochrome P450 enzyme family is essential for medication removal through metabolic biotransformation. table 1 displays the log-Kp values for all tested medications, which range from -8.0 to 1.0.

Lipophilicity and drug-likeness

Lipinski's rule-of-five

If any of the four parameters listed below are not met, the medicine will most likely be created orally, according to Lipinski's rule. The ADME test was passed by every chemical. This shows that molecules resembling pharmaceuticals may be synthesized using the series (table 2).

Medicinal chemistry

Bread includes unnatural substances that cause inappropriate reactions with all protein receptors, significantly affecting life. The SA score was less than five, indicating that they did well. The synthetic accessibility score (AS) ranges from 1 to 10. The majority of librarians have compatibility scores of less than four on the SA scale. A biology-based approach supports medical practitioners in determining the necessary elements for biological optimization. All of them are appropriate for biological optimization. According to the Swiss ADME prediction, phytocompounds have the greatest ratio of any parameter, indicating their potential for chemotherapeutic use.

Docking analysis

We used Chem 3D and Chem-draw Ultra 12.0 pro for docking studies in order to minimise ligand energy utilising PYRX. In order to determine their effectiveness, the docking study examined the molecular interactions between the receptor proteins Cox-1 (PDB: 2OYE) and Cox-2 (1CX2). The coordinate structure of these receptors was taken from the PDB and gradually added to PYRX at a resolution of 2.70 in order to finish docking. PYRX algorithm determined which chemical optimally interacts with the receptor. The optimal drug was chosen based on docking scores and the binding affinity of the ligand with the greatest affinity for the receptor. The results were assessed based on binding compatibility. According to the dock scoring, three compounds had the best score indicating that they have effective chemotherapy potential. The discovery studio's best postures were represented in 2D and 3D, with protein-ligand interactions depicted in fig. 3 and 4.

DISCUSSION

Despite having a lengthy folklore history, *D. bipinnata's* pharmaceutical value for the central nervous system is unclear. In order to do this, the current study aims to thoroughly examine the third plant's neurological effects. This study examined the pharmacological applications of *D. bipinnata* in the treatment of such neurological diseases using mouse model behavioural assessments, such as the elevated plus maze test (EPM), hole board test (HBT), hole cross test (HCT), and open field test (OFT), which evaluate anxiety (Ali Reza *et al*., 2018; Cheng *et al*., 2024). The thiopental sodium-induced sleeping test was also used to evaluate the effect of *D. bipinnata* on the initiation and duration of sleep. The duration of time spent in the open arm during the EMP test was significantly increased by the anxiolytic diazepam. Mice administered EEDBR showed a similar pattern, spending much longer time in the open arm. The GABA agonist diazepam, which raises GABA levels in the brain and has anxiolytic effects, served as the positive standard in all of the trials. A popular behavioral test for mice, the EPM test has shown promise in pinpointing the parts of the brain and the mechanisms behind anxiety-related behaviors. It has also been shown to be helpful in evaluating the anti-anxiety benefits of pharmaceutical drugs and steroid hormones. The findings are corroborated by another study that increased open arms response (Saivasanthi *et al*., 2011; Zou *et al*., 2024).

Another anxiolytic effect was testified by OPF test. The open field test is a common measure of exploratory behavior and general activity in mice (Reza *et al*., 2023). Mice showed strong anxiolytic activity at the highest dose 400mg/kg in the open arms and in Diazepam group. EEDBR treated mice lowered frequency of line crossing in dose dependant manner. Grooming and rearing behavior was significantly less in EEDBR treatment similar to standard drug but diazepam has a greater anxiolytic effect. Mice with *D. bipinnata* extract (400mg/kg) spent more time in the center compared to control. Another study of *Ficus Benghalensis* extract demonstrated similar behavior in rats (Malik *et al*., 2020). Diazepam significantly improves response in line crossing, grooming, rearing and time spent in the center of apparatus. However, 400mg/kg dose has same time in center of apparatus as compared to diazepam group. Anxious behavior can be elicited by the Hole Board Test, a behavioral test intended to assess an animal's attitude towards novel situations. It is a reliable predictor of animal emotional behavior, according to studies. *D. bipinnata* demonstrated significant dose-dependent anxiolytic effects in hole board test (HBT). Mice showed a substantial increase in heads dips (62.66±2.5) at 400mg/kg as compared to the control (32.66±2.51), indicating a decrease in anxiety and calmness. Similarly, standard group has more significant effect in head dips (52.6±2.5) as compared to control and EEDBR treated groups. Either an irregularity brought on by glutamatergic, serotonergic, GABA-ergic, or noradrenergic transmission, or aberrant activation of neurotransmitters like serotonin, dopamine, or GABA receptors, can produce anxiety (Trifu *et al*., 2021). It was

demonstrated that EEDBR may indicate chemical transfer to produce anxiolytic effects. The hole cross test, most explorative method also showed dose-dependent result in lowering crossing holes. Plant extract lowered locomoter activity at 1st observation 930 minutes) and evident till 5th observation (120 minutes). Diazepam has more significant lower holes crossed by mice. Similar CNS depressant activity reported by *Pterocarpus indicus* in swiss albino mice at 250 and 500mg/kg dose (Rajib *et al*., 2021). *EEDBR* demonstrated significant CNS depressant activities effects in the HCT. Mice showed a substantial decrease in hole cross at the 400mg/kg as compared to, indicating a decrease in anxiety. Overall, the studied plant extract demonstrated significant CNS depressant activities effects in the HCT.

In the TPSIST, mice showed strong CNS depressant effects that significantly reduced sleep latency and improved sleep duration at the highest dose when compared to the control group. It binds to the GABA receptor complex and causes postsynaptic neurons to become hyperpolarized by GABA (Gan *et al*., 2024). By extending the time of the chloride channel opening, it increases GABA activity and allows chloride to enter the cell. However, thiopental has the ability to inhibit glutamate receptors that are excitatory (Cao *et al*., 2024; Khatun, 2023). At 400mg/kg dose latency time was 9.3±2.08 and 14.6±1.52 while sleeping duration increased 51.66±22.51 and 75±23.0 respectively as compared to control latency time was 49.3±2.51 and slept for 63.33±251. Diazepam has more significant effect in latency onset (18±1) and sleeping duration (70±1.0). Similar antidepressant activity observed by ethanolic extract of *Nypa fruticans* at different doses (Lubaba, 2023).

It has been shown that EEDBR's anxiolytic action may result from their capacity to alter neurotransmitter systems, including the dopaminergic, GABAergic and serotonergic systems. They play a part in controlling anxiety and other emotional systems (Hoque *et al*., 2021). According to earlier research, benzodiazepines work by attaching to the GABA receptor independently of the GABA receptor's binding site to produce their calming effects (Whiting, 2003). By hyperpolarising post-synaptic neuronal cells, thiopental sodium produces a synergistic effect when it binds to the GABA receptor complex (Fernández *et al*., 2004). Mice were given injections of diazepam or thiopental sodium to test the function of GABA-ergic systems in EEDBR-induced sedation. Overall, this study's results supported the use of pharmaceuticals to treat mental disorders like anxiety. However, further investigation is needed to determine the function of additional isolated compounds for the stated activity, as it is yet unclear if particular components generated these effects. Mice were given injections of diazepam or thiopental sodium to test the function of GABA-ergic systems in EEDBR-induced sedation. Overall, this study's results supported the use of pharmaceuticals to treat mental disorders like anxiety. However, further investigation is needed to determine the function of additional isolated compounds for the stated activity, as it is yet unclear if particular components generated these effects (Müller, 2019). The cycloxygenase-2 enzyme is primarily responsible for anxiety and depression, and selective COX-2 inhibitors are more efficient means of preventing the pain-stimulating activity. Cox-2 drugs do, however, have a number of drawbacks. PYRX docking scores of substances favour protein receptors in our investigation. Thus, molecular docking validated EEDBR's antidepressant efficacy. Overall, this study's results supported the use of pharmaceuticals to treat mental disorders like anxiety.

CONCLUSION

From the foregoing experiment, it may be inferred that *Desmostacchya bipinnata* ethanolic extract has strong central nervous system depressant properties. It was evident that the results in the experiment above were statistically significant at all of the dosages utilized. To investigate the extract's potential for therapeutic use in the future and to comprehend the molecular mechanisms underlying its pharmacological activity, more research must be done to isolate the active constituent that is responsible for the CNS depressant activity.

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REFERENCES

Ali MS, Dash PR and Nasrin M (2015). Study of sedative activity of different extracts of Kaempferia galanga in Swiss albino mice. *BMC Complement. Altern. Med.*, **15**: 1-5.

Ali Reza A, Hossain MS, Akhter S, Rahman MR, Nasrin MS, Uddin MJ, Sadik G and Khurshid Alam A (2018). *In vitro* antioxidant and cholinesterase inhibitory activities of *Elatostema papillosum* leaves and correlation with their phytochemical profiles: A study relevant to the treatment of Alzheimer's disease. *BMC Complement. Altern. Med.*, **18**: 1-8.

Alqudah AA, Al Hawamdeh B, Ali D, Alfarrayeh I, Algataitat B, Al-Mobideen OK and Alhawatema M (2023). Comparison of antibacterial and antioxidant activities of ethanolic extracts of four plant species selected from south of Saudi Arabia. *Pharmacog. J.*, **15**(4): 691-696.

Ammor K, Mahjoubi F, Bousta D and Chaqroune A (2020). Ethnopharmacological survey of medicinal plants used in the traditional treatment of kidney stones

realized in Fez-Morocco. *Ethnobot. Res. Appl.*, **19**: 1-12.

Batiha GES, Teibo JO, Wasef L, Shaheen HM, Akomolafe AP, Teibo TKA, Al-Kuraishy HM, Al-Garbeeb AI, Alexiou A and Papadakis M (2023). A review of the bioactive components and pharmacological properties of Lavandula species. *N-S Arch. Pharmacol.*, **396**(5): 877-900.

Bibi S, Ahmad MSA, Hameed M and Alvi AK (2024). Water conservation strategies in big cordgrass (*Desmostachya bipinnata* L.) for ecological success in hyper-arid and saline-arid environments. *Arid Land Res. Manag.*, **38**(1): 81-96.

Cao X, Wang Z, Chen Y and Zhu J (2024). Childhood maltreatment and resting-state network connectivity: The risk-buffering role of positive parenting. *Dev. Psychopathol.*, pp.1-12.

Cheng X, Huang J, Li H, Zhao D, Liu Z, Zhu L, Zhang Z and Peng W (2024). Quercetin: A promising therapy for diabetic encephalopathy through inhibition of hippocampal ferroptosis. *Phytomedicine*, **126**: 154887.

Farzeen I, Nazir MM, Muzammil S, Zafar S, Yeni DK and Ashraf A (2024). Bergamottin a bioactive compound unveiled: Exploring its potential in disease managments and in-silico insights. *Phytochem. Rev.*, pp.1-21.

Feng D, Li P, Xiao W, Pei Z, Chen P, Hu M, Yang Z, Li T, Xia Z and Cui H (2023). N6-methyladenosine profiling reveals that Xuefu Zhuyu decoction upregulates METTL14 and BDNF in a rat model of traumatic brain injury. *J. Ethnopharmacol.*, **317**: 116823.

Fernández S, Wasowski C, Paladini AC and Marder M (2004). Sedative and sleep-enhancing properties of linarin, a flavonoid-isolated from *Valeriana officinalis. Pharmacol. Biochem. Behav.*, **77**(2): 399-404.

Gan Y, Huang H, Wu X and Meng M (2024). What doesn't kill us makes us stronger: Insights from neuroscience studies and molecular genetics. *Curr. Opin. Behav. Sci.*, **59**: 101431.

Goni O, Khan MF, Rahman MM, Hasan MZ, Kader FB, Sazzad N, Sakib MA, Romano B, Haque MA and Capasso R (2021). Pharmacological insights on the antidepressant, anxiolytic and aphrodisiac potentials of *Aglaonema hookerianum* Schott. *J. Ethnopharmacol.*, **268**: 113664.

Hamida R, Ouahiba B, Khaled B, Tarek B, Felix T, Pierre T and Selma D (2024). A novel anti-candidiasis cream formulation based on *Melissa officinalis* and *Lavandula stoechas* essential oils synergism. *J. Essent. Oil Bear. Plants*, **27**(2): 510-524.

Heissel A, Heinen D, Brokmeier LL, Skarabis N, Kangas M, Vancampfort D, Stubbs B, Firth J, Ward PB and Rosenbaum S (2023). Exercise as medicine for depressive symptoms? A systematic review and meta-analysis with meta-regression. *Br. J. Sports Med.,* **57**(16): 1049-1057.

Hoque MA, Ahmad S, Chakrabarty N, Khan MF, Kabir MSH, Brishti A, Raihan MO, Alam AK, Haque MA and Nasrin MS (2021). Antioxidative role of palm grass rhizome ameliorates anxiety and depression in experimental rodents and computer-aided model. *Heliyon*, **7**(10): 1-10.

Jaouani M, Maouni S, Ettakifi H, Mars N, Taheri FZ, El Abboudi J, Haddad O, Saidi R, Lamrani Z and Maouni A (2024). Molecular, biomedical and phytosanitary biodiversity of Lavandula stoechas: A vulnerable and underexploited medicinal plant in Morocco. *Sci. Afr.*, e02296.

Kang L, Gao XH, Liu HR, Men X, Wu HN, Cui PW, Oldfield E and Yan JY (2018). Structure activity relationship investigation of coumarin chalcone hybrids with diverse side-chains as acetylcholinesterase and butyrylcholinesterase inhibitors. *Mol. Divers*, **22**: 893-906.

Khatoon MM, Khatun MH, Islam ME and Parvin MS (2014). Analgesic, antibacterial and central nervous system depressant activities of *Albizia procera* leaves. *Asian Pac. J. Trop. Biomed.*, **4**(4): 279-284.

Khatun A (2023). Evaluation of central nervous system (cns) depressant activity of methanolic extract of *Enhydra fluctuans* Lour. in mice. *Clin. Phytosci.*, **4**(5): 1-7.

Kong F, Xu Z, Yang G, Jia Q, Mo F, Jing L, Luo J, Jin H and Cai X (2024). Microelectrode arrays for detection of neural activity in depressed rats: Enhanced theta activity in the basolateral amygdala. *Cyborg. Bionic Systems*, **5**: 0125.

Kouémou NE, Wanyu BY, Njapdounke JK, Pale S, Noubissi PA, Manyi RF and Taiwe GS (2024). Anxiolytic effects of *Dichrocephala integrifolia* leaf aqueous extract on alcohol withdrawal-induced anxiety in mice: Involvement of the GABAergic pathway. *Sci. Afr.*, **23**: e02124.

Kozubski W, Ong K, Waleszczyk W, Zabel M and Dorszewska J (2021). Molecular factors mediating neural cell plasticity changes in dementia brain diseases. *Neural Plast*, **1**: 8834645.

Li W, Zhao Z, Chen D, Peng Y and Lu Z (2022). Prevalence and associated factors of depression and anxiety symptoms among college students: A systematic review and meta-analysis. *J. Child Psychol. Psychiatry*, **63**(11): 1222-1230.

Lubaba RR (2023). Analgesic and CNS depressant activities of methanolic shell extract of *Nypa fruticans* Wurmb fruit in Swiss albino mice (Thesis). School of Pharmacy, Brac University, Brac University, Dhaka, Bangladesh pp. 28-34.

Malik H, Javaid S, Fawad Rasool M, Samad N, Rizwan Ahamad S, Alqahtani F and Imran I (2020). Amelioration of scopolamine-induced amnesic, anxiolytic and antidepressant effects of *Ficus benghalensis* in behavioral experimental models. *Medicina*, **56**(3): 144.

Muller N (2019). COX-2 inhibitors, aspirin and other potential anti-inflammatory treatments for psychiatric disorders. *Front. Psychiatry*, **10**: 375.

Nawrin K, Billah MM, Jabed MSU, Roy A, Ahmad A and Islam MN (2015). Antipyretic, antidiabetic, thrombolytic and CNS depressant potential of ethanol extract of *Crotalaria verrucosa* L. leaves. *Am J Biomed Sci.*, **7**(4): 198-204.

Nazir MM, Inam S, Ijaz MU, Zafar N, Yeni DK, Asad F, Farzeen I and Ashraf A (2024). *In vivo* and *in silico* elucidation of possible potential and mechanisms involved in the analgesic action of ethanolic extract of *Lavandula stoechas*. *J. Pharm. Pharmacol.*, **76**(9): 1178-1198.

Ndhlala AR, Işık M, Kavaz Yuksel A and Dikici E (2024). Phenolic content analysis of two species belonging to the lamiaceae family: Antioxidant, anticholinergic and antibacterial activities. *Molecules*, **29**(2): 480.

Ormel J, Hollon SD, Kessler RC, Cuijpers P and Monroe SM (2022). More treatment but no less depression: The treatment-prevalence paradox. *Clin. Psychol. Rev.*, **91**: 102111.

Putta SK, Koteshwara K, Kamath V and Aswatha Ram H (2023). Desmostachya bipinnata: A focused review on ethnobotany, phytoconstituents and biological activities. *Rasayan J. Chem.*, **16**(2): 686-691.

Rajib AH, Rahman M, Majumder S, Akter F, Islam F, Shahriar M and Alam J (2021). Pre-clinical investigation of analgesic, anti-diarrheal and CNS depressant effect of *Pterocarpus indicus* in Swiss albino mice. *Jordan J. Pharm. Sci.*, **14**(1): 85-94.

Reza AA, Sakib MA, Nasrin MS, Khan J, Khan MF, Hossen MA, Ali MH and Haque MA (2023). *Lasia spinosa* (L.) thw. attenuates chemically induced behavioral disorders in experimental and computational models. *Heliyon*, **9**(6): 1-10.

Saivasanthi V, Gowthamigoud SK, Aakruthi SR, Gupta A and Rao A (2011). Evaluation of *Caralluma fimbrita* for analgesic, anti inflammatory and anxiolytic activities. *Int. J. Pharm.*, **1**(1): 40-45.

Shaheen H, Qureshi R, Qaseem MF and Bruschi P (2020). The fodder grass resources for ruminants: A indigenous treasure of local communities of Thal desert Punjab, Pakistan. *PLoS One*, **15**(3): e0224061.

Shrestha A, Pradhan R, Ghotekar S, Dahikar S and Marasini B (2021). Phytochemical analysis and anti-microbial activity of *Desmostachya bipinnata*: A review. *J. Med. Chem. Sci*, **4**: 36-41.

Trifu S, Drăgan-Serban F and Jianu E (2021). Neurotransmitters, neuromodellers, anatomical structures involved in psychiatric pathologies. *Recent Pharm*, **147**: 144-174.

Uddin MJ, Ali Reza A, Abdullah-Al-Mamun M, Kabir MS, Nasrin MS, Akhter S, Arman MSI and Rahman M A (2018). Antinociceptive and anxiolytic and sedative effects of methanol extract of *Anisomeles indica*: An experimental assessment in mice and computer aided models. *Front. Pharmacol.*, **9**: 246.

Vivekanandarajah S and Rajamanoharan P (2021). Bioactivities of *Desmostachya bipinnata* (L.) Stapf. *Plant Biotechnol. Persa.*, **3**(1): 18-25.

Wang L, Wang X, Deng L, Zhang H, He B, Cao W and Cui Y (2022). Pexidartinib (PLX3397) through restoring hippocampal synaptic plasticity ameliorates social isolation-induced mood disorders. *Int. Immunopharmacol.*, **113**: 109436.

Wang W and Peng Y (2023). Mechanochemical organic synthesis in a rotary evaporator beyond conventional application: Proof-of-concept reactions. *Synth. Commun.*, **53**(9): 625-639.

Whiting PJ (2003). GABA-A receptor subtypes in the brain: A paradigm for CNS drug discovery? *Drug Discov. Today*, **8**(10): 445-450.

Zajkowska Z, Walsh A, Zonca V, Gullett N, Pedersen G A, Kieling C, Swartz JR, Karmacharya R, Fisher HL, and Kohrt BA (2021). A systematic review of the association between biological markers and environmental stress risk factors for adolescent depression. *J. Psychiatr. Res.*, **138**: 163-175.

Zou GJ, Chen ZR, Wang XQ, Cui YH, Li F, Li CQ, Wang LF and Huang Fl (2024). Microglial activation in the medial prefrontal cortex after remote fear recall participates in the regulation of auditory fear extinction. *Eur. J. Pharmacol.*, 176759.