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Pharmacological Evaluation of Anticonvulsant and Anxiolytic Effects of *Mitragyna speciosa* Leaf Alkaloid-Enriched Fraction through Experimental and Computational Studies

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The present study aimed to evaluate the anticonvulsant and anxiolytic potential of the alkaloid-enriched leaf fraction of *Mitragyna speciosa* (AEMS) using experimental and computational approaches. Powdered leaves were extracted by Stas-Otto method to obtain the alkaloid-enriched fraction, followed by LC-MS and preliminary phytochemical analysis using standard methods. AEMS was administered at doses of 50 mg/kg and 100 mg/kg and its effects were assessed using *in vivo* models over 15 days. Statistical analysis was performed using one-way ANOVA followed by Dunnett's test. *In vitro* antioxidant assays and *in silico* molecular docking studies were conducted to evaluate interactions with the $GABA_A$ -BZD receptor. LC-MS and phytochemical screening confirmed the presence of alkaloids, *viz.* mitragynine, 7-hydroxymitragynine and corynoxine B. AEMS demonstrated significant (*$p < 0.05$) anticonvulsant and anxiolytic activities, with the 50 mg/kg dose exhibiting greater efficacy. The antioxidant activity was reported to be comparatively lesser (IC_{50} = 399.93 ± 1.35 µg/mL) than the ascorbic acid standard (IC_{50} = 49.6 ± 6.55 µg/mL) by DPPH method. Docking studies revealed strong binding affinities for all the three compounds wherein, 7-hydroxymitragynine demonstrated profound binding affinity (-7.7 kcal/mol), comparable to diazepam (-8.8 kcal/mol), suggesting GABAergic involvement. These findings indicate that AEMS possesses significant anticonvulsant and anxiolytic activity and may be useful in managing convulsions and anxiety.

Keywords: *Mitragyna speciosa*, Alkaloid-enriched fraction, Anxiolytic, Anti-convulsant, $GABA_A$-BZD receptor.

INTRODUCTION

The use of plants in healthcare has been documented from ancient times and continues to play an important role in modern therapy. Their extensive and diverse applications have led to the discovery of a wide range of phytochemicals, many of which have contributed to the development of numerous therapeutic agents used in the prevention and treatment of various diseases. Furthermore, ongoing research into medicinal plants continues to uncover novel bioactive compounds, reinforcing their significance as a valuable source for future drug discovery and development [1-3].

Epilepsy is a chronic neurological disorder characterized by recurrent seizures and affects over 50 million people worldwide, with nearly 30% of patients exhibiting resistance to available therapies [4,5]. Anxiety frequently coexists with epilepsy, further increasing disease burden and reducing quality of life [6]. Alterations in GABAergic neurotransmission and oxidative stress have been implicated in the pathogenesis of both conditions [7-11]. Despite the availability of conventional antiepileptic and anxiolytic drugs, their clinical use is often limited by adverse effects and treatment resistance [12, 13].

Mitragyna speciosa, the most common of the *Mitragyna* species, is a tropical evergreen tree native to Southeast Asia, belonging to the coffee family Rubiaceae. It is particularly found in Cambodia, Thailand, Indonesia, Malaysia, Myanmar and Papua New Guinea, where it is commonly known as Kratom [14-17]. The alkaloids mitragynine and 7-hydroxymitragynine are predominantly found in this plant and are largely responsible for its various biological activities such as analgesic, antinociceptive, antidepressant, *etc*. [14]. It also contains various

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other alkaloids, flavonoids, phenolic acids, saponins and triterpenoids and glycoside derivatives [18].

Although numerous studies have been conducted on this plant using various extracts (ethanolic, methanolic, aqueous) the anticonvulsant and anxiolytic potential of its alkaloid-enriched fraction, particularly, given its predominance of alkaloids, has not been thoroughly explored. Therefore, the present study aims to address this by evaluating its anticonvulsant and anxiolytic activities using integrated experimental and computational methods.

EXPERIMENTAL

The analytical-grade chemical and solvents were used and sourced from the reputed chemicals suppliers. Fresh biochemical reagents were prepared for the experiments. Pentylenetetrazole (PTZ) was procured from Otto Chemie Pvt. Ltd., while diazepam was obtained from Abbott Healthcare Ltd. Analytical grade *n*-hexane, chloroform, petroleum ether and methanol were purchased from Molychem, India.

Plant material: Dried leaf powder of *M. speciosa* was supplied by Bio Med Ingredients, Verna, Goa (Batch No.: BMIS20250326), accompanied by a botanical declaration authenticating the plant species.

Preparation of alkaloid-enriched fraction of *M. speciosa* (AEMS): For the preparation of AEMS, 500 g of *M. speciosa* dried leaf powder was weighed and defatted with *n*-hexane by refluxing six times for 90 min each. The solvent was decanted at the end of each cycle. The dried marc obtained was then moistened with ammonia and dried overnight. The dried powder was then refluxed three times using chloroform for 90 min each. The solvent was filtered, collected in a porcelain dish and then evaporated to dryness, yielding the alkaloid-enriched fraction of *M. speciosa* (AEMS) [19].

Phytochemical analysis

Qualitative screening: The alkaloid-enriched fraction of *M. speciosa* (AEMS) was subjected to a preliminary quantitative investigation for the detection of phytochemicals such as alkaloids, carbohydrates, proteins, glycosides, flavonoids, steroids, tannins and saponins, using the standard methods [20,21].

LC-MS analysis: Liquid chromatography-mass spectrometry (LC-MS) analysis of AEMS was performed using a Shimadzu HPLC system equipped with an LC-10ADVp pump, PDA/UV detector, SIL-HT autosampler, DGU-12A degasser and CTO-10AVp column oven. Data acquisition and processing were carried out using LC Solution software (Version 1.25 SP4). LC–MS/MS separation was achieved using a Waters X Bridge C18 column (50 × 4.6 mm, 3.5 µm particle size). The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (acetonitrile, MS grade), delivered at a flow rate of 1.0 mL/min under gradient elution conditions. The sample diluent comprised a mixture of methanol, acetonitrile, water, 0.1% formic acid and trifluoroacetic acid (TFA). The gradient program was set as follows: 0.01 min/15% B, 6.00 min/75% B, 8.00 min/75% B, 11.00 min/15% B and 15.00 min/15% B, with a total run time of 15.01 min. Detection was carried out using a PDA/UV detector.

In vitro antioxidant activity

DPPH radical scavenging assay: A DPPH solution (0.004%) was prepared in methanol. Different concentrations of AEMS and standard (ascorbic acid) solutions (25-400 µg/mL) were prepared. To 1 mL of AEMS, 2 mL of DPPH solution was added in a test tube. All test tubes were shaken and incubated in the dark at room temperature for 30 min. The absorbance was measured at 517 nm using UV-visible spectrophotometer. Change in colour of DPPH from deep purple DPPH to yellow indicates that the plant extract or standard has successfully neutralised free radicals. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The DPPH scavenging activity was calculated using the following equation [22]:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Hydrogen peroxide (H_2O_2) scavenging assay: Different concentrations of AEMS and standard (ascorbic acid) solutions (25-400 µg/mL) were prepared. Ferrous ammonium sulphate (0.25 mL) and 1.5 mL of AEMS or standard ascorbic acid was added in the test tubes. Hydrogen peroxide (62.5 µL) was then added and the solutions were incubated for 5 min in dark followed by 1,10-phenanthroline (1.5 mL) and then incubated for 10 min. The absorbance was measured at 510 nm using UV-visible spectrophotometer. Hydrogen peroxide free radical scavenging activity was calculated using the formula [23]:

$$H_2O_2 \text{ scavenging activity (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Ferric reducing antioxidant power assay (FRAP): Stock solution of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-striazine) solution in 40 mM HCl and 20 mM $FeCl_3$ solution. The working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ and 2.5 mL $FeCl_3$ solution. This working solution (2 mL) was added to the different concentrations of AEMS and standard (ascorbic acid) solutions (25-400 µg/mL) and placed in the dark for 30 min. The absorbance was measured at 593 nm using UV-visible spectrophotometer. The absorbance is directly proportional to the reducing power, hence higher the absorbance, higher is the antioxidant activity [24,25].

In vivo studies

Ethics committee approval: All experimental protocols were reviewed and accepted by the Institutional Animal Ethics Committee (IAEC) prior to commencement of the experiment (GCP/IAEC/2025/08).

Dose selection: Previous oral toxicity studies on the alkaloid-enriched fraction of *M. speciosa* demonstrated its safety up to 1000 mg/kg; therefore, doses of 50 and 100 mg/kg were selected for the present study [26].

Animals used: Male Wistar albino rats (n = 24; 150-250 g) were obtained from LACSMI Biofarms Pvt. Ltd., Pune, India (CCSEA Registration No. 277/PO/RcBt/S/09/CCSEA). Animals were maintained under controlled environmental conditions (25 ± 2 ºC, 55 ± 10% relative humidity and a 12 h light/dark cycle) with unrestricted access to standard pellet

feed and water. Following a one-week acclimatization period, the animals were used for the study. All experimental protocols complied with the guidelines of the Committee for the Control and Supervision of Experiments on Animals (CCSEA), Government of India.

Treatment groups: For the evaluation of anticonvulsant and anxiolytic activity of AEMS, the rats were divided into 4 groups of 6 animals each. The control group was administered propylene glycol:Tween-80:distilled water (4:1:4), the standard with diazepam 2 mg/kg, while the test groups were administered AEMS 50 mg/kg and 100 mg/kg, *via* the oral route, for 15 days. The behavioural tests for anxiolytic activity *viz.* elevated plus maze (EPM), light and dark model (LDM) and open field test (OFT) were conducted on day 1, day 7 and day 14, 1 h after dose administration. The behavioural test for anticonvulsant activity *viz.* PTZ (pentylenetetrazol)-induced convulsions was conducted on day 15, 1 h after dose administration.

Behavioural model for anticonvulsant activity

PTZ-induced convulsions: Convulsions were induced using pentylenetetrazol (PTZ), a chemoconvulsant commonly used to evaluate anticonvulsant activity. After 1 h of treatment, PTZ was administered intraperitoneally at a dose of 70 mg/kg to induce seizures. Following PTZ administration, each animal was placed individually in an observation cage and behaviour was monitored continuously for 30 min and recorded using a camera. Latency to onset of seizures, number of animals exhibiting convulsions, duration of convulsions was recorded manually and percentage protected from seizures and lethality was calculated [6,27,28].

Behavioural models for anxiolytic activity

Elevated plus maze (EPM): The EPM consisted of two open arms and two enclosed arms of the same dimensions (50 × 10 × 40 cm), crossing each other forming a 'plus' shape with a central platform. The maze is elevated at a height of 50 cm above ground level. After 1 h of treatment, the rats were placed individually at the centre facing one of the open arms and the behaviour was recorded for 5 min using Smart v3.0.02 software. The percent open arm entries (%OAE) and percent time spent in open arms (%TSOA) were measured as indices of anxiolytic activity [29-31].

Light and dark model (LDM): The light-dark box apparatus consisted of two equal-sized compartments (40 × 40 × 40 cm each) connected by a 6 × 6 cm opening in the partition wall. The illuminated compartment was painted white and lit by a 40 W bulb, while the dark compartment was painted black. One hour after treatment administration, each rat was placed individually in the centre of the illuminated compartment, and its behaviour was recorded for 5 min using a video camera. The number of entries into the light compartment (NEL) and the time spent in the light compartment (TSL) were recorded as indices of anxiolytic activity [29-31].

Open field test (OFT): Locomotor activity was assessed using an actophotometer consisting of a central chamber (44 × 33 × 20 cm) equipped with photocells positioned on opposite walls. One hour after treatment administration, rats were individually placed in the chamber and their activity was recorded for 5 min using ATM-3 software. The parameters evaluated included distance travelled (DT), resting time (RT), ambulatory time (AT) and stereotypic time (ST) [29-31].

Statistical analysis: Results of all the above-mentioned models were statistically analysed using one-way ANOVA followed by Dunnett's post hoc test, in which the results obtained from experimental samples were compared with those of the control. All the results were expressed as mean ± SEM (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001) (n = 6).

In silico docking simulation studies

Preparation of the protein: The 3D structure of protein was obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank database (RCSB) bearing PDB ID: 6X3X ($GABA_A$ -BZD receptor for anxiety). Files were downloaded in PDB format. The protein was prepared by adding hydrogen and removing water molecules. The X, Y, Z coordinates were recorded from BIOVIA Discovery Studio v.25.1.0.24284 [32].

Preparation of the ligand: The 3D structures of the compounds identified from *M. speciosa* leaves were obtained from PubChem. The structures were downloaded in SDF format and these files were then converted to PDB format using an online SMILES translator. Both ligand and protein PDB files were converted to PDBQT using AutoDockTools 1.5.7. Diazepam was used as the standard for comparison [32].

Docking and visualisation of protein-ligand interactions: The molecular docking was carried out using AutodockVina. AutoDock tools was used to transform the input ligands into the PDBQT format in AutoDockVina. The grid box was configured with the grid dimensions (20 × 20 × 20) and the X, Y and Z coordinates of 145.54350 Å, 123.61150 Å and 122.43005 Å respectively. The docked ligand-protein complexes were visualised with PyMOL and interactions were analysed using BIOVIA Discovery Studio. The binding affinity of each phytoconstituent, was evaluated using a negative score, with a larger negative energy indicating a better binding affinity [32].

RESULTS AND DISCUSSION

The alkaloid-enriched fraction of *M. speciosa* (AEMS) was obtained with a percentage yield of 4.33% (w/w). Preliminary qualitative phytochemical screening confirmed the presence of multiple phytoconstituent classes, as summarized in Table-1. Further phytochemical characterization by LC-MS analysis revealed the presence of three major alkaloids, namely mitragynine (*m.w.*: 398.5 g/mol), 7-hydroxymitragynine (*m.w.*: 414.5 g/mol) and corynoxine B (*m.w.*: 384.47 g/mol), thereby confirming the alkaloid-rich nature of the fraction (Fig. 1).

TABLE-1
PRELIMINARY PHYTOCHEMICAL ANALYSIS OF AEMS

Phyto-constituents	Observation	Phyto-constituents	Observation
Alkaloids	Positive	Flavonoids	Negative
Carbohydrates	Negative	Steroids	Negative
Proteins	Negative	Tannins	Negative
Glycosides	Negative	Saponins	Negative

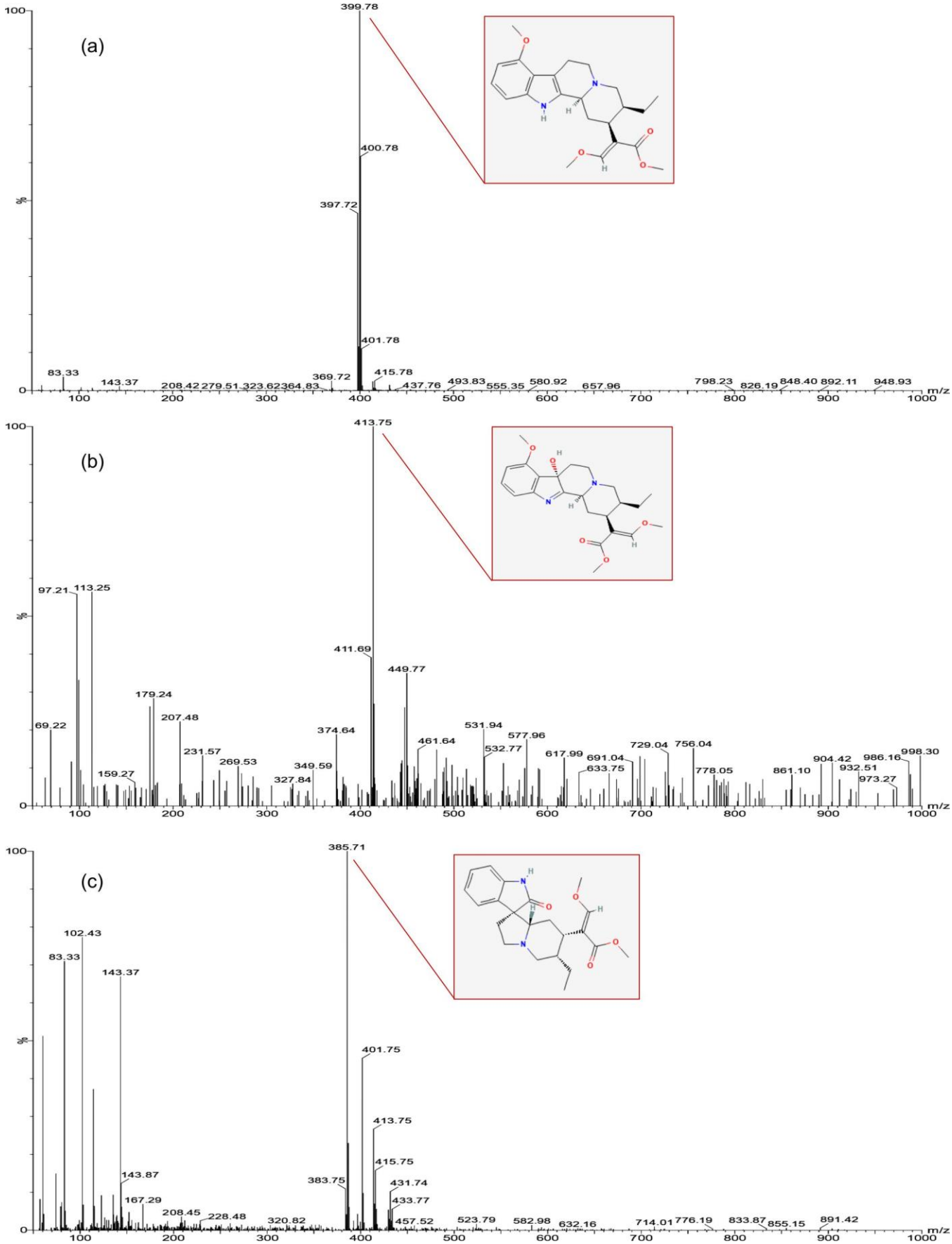

Fig. 1. LC-MS spectra of (a) mitragynine, (b) 7-hydroxymitragynine and (c) corynoxine B

***In vitro* antioxidant activity:** The *in vitro* antioxidant activity of AEMS, evaluated using DPPH, H_2O_2 scavenging, and FRAP assays, is summarized in Table-2. AEMS exhibitted a ferric reducing antioxidant capacity of 46.52 ± 0.27 mg AAE/g and displayed mild to moderate free radical scavenging properties, which may contribute to protection against oxidative stress-mediated neuronal damage.

TABLE-2
in vitro ANTIOXIDANT ASSAYS OF AEMS

Samples	IC_{50}	
	DPPH (µg/mL)	H_2O_2 (µg/mL)
Standard (ascorbic acid)	49.6 ± 6.55	37.06 ± 6.57
AEMS	399.93 ± 1.35	410.77 ± 16.34

Values are expressed as mean ± SEM (n=3)

In vivo studies

Anticonvulsant study

PTZ-induced convulsions: The anticonvulsant effects of AEMS in the PTZ-induced seizure model are shown in Table-3. Administration of the alkaloid-enriched fraction of *M. speciosa* reduced seizure susceptibility, as reflected by delayed seizure onset, decreased seizure duration and improved survival. AEMS at 50 mg/kg completely prevented PTZ-induced convulsions, affording 100% protection against both seizures and lethality. In contrast, treatment with AEMS at 100 mg/kg resulted in partial protection, with 33.33% protection against seizures and 83.33% protection against lethality, accompanied by a reduction in seizure duration (**$p < 0.01$).

Anxiolytic activity: The anxiolytic effects of AEMS evaluated through EPM, LDM and OFT are demonstrated in Figs. 2-4.

Effects of AEMS in elevated plus maze: In the EPM test, treatment with AEMS at 50 mg/kg significantly increased the percentage of open arm entries (%OAE; ***$p < 0.001$) and percentage time spent in the open arms (%TSOA; **$p < 0.01$) on day 7 compared with the control group. On day 14, both AEMS 50 mg/kg (**$p < 0.01$) and AEMS 100 mg/kg (*$p < 0.05$) increased open arm exploration relative to the control (Fig. 2). Rats treated with AEMS exhibited greater open arm preference and prolonged open arm residence time, reflecting reduced anxiety-like behaviour comparable to that observed with standard diazepam treatment.

Effects of AEMS in light and dark model: The anxiolytic activity of AEMS in the light-dark box test is presented in Fig. 3. Treatment with AEMS at 50 mg/kg significantly increased the time spent in the light compartment on day 14 (*$p < 0.05$) compared with the control group. Changes in the number of entries into the light compartment were less pronounced. The increased preference for the illuminated compartment is consistent with an anxiolytic-like effect of AEMS.

Effect of AEMS in open field test: The effects of AEMS on locomotor activity in the open field test are shown in Fig. 4. Data are expressed as mean ± SEM (n = 6), with *$p < 0.05$ considered statistically significant compared with the control group according to Dunnett's post hoc test. Treatment with AEMS at both 50 and 100 mg/kg significantly reduced the distance travelled (*$p < 0.05$) and increased resting time (*$p < 0.05$) across all three experimental days. The reduction in locomotor activity, coupled with increased resting behaviour, is indicative of sedative and central nervous system depressant properties, which may contribute to the anxiolytic effects observed in the elevated plus maze and light-dark box models. These behavioural effects were comparable to those produced by standard diazepam [30,31]. The *in vivo* findings further indicate that AEMS at 50 mg/kg exerted greater anticonvulsant and anxiolytic activity than the 100 mg/kg dose. This response pattern is consistent with an inverted U-shaped dose-response relationship, wherein maximal efficacy occurs at

TABLE-3
THE EFFECTS OF AEMS ON PTZ (PENTYLENETETRAZOL)-INDUCED CONVULSIONS

Treatment	No. of animals exhibiting seizures	Latency (s)	Duration of seizures (s)	Number of animals survived	Protection against seizures (%)	Protection against lethality (%)
Control	6/6	4.17 ± 2.97	1018.67 ± 115.05	0/6	0	0
Diazepam	4/6	287.75 ± 141.32**	386.5 ± 201.93*	6/6	33.33	100
AEMS 50	0/6	NC#	NC#***	6/6	100	100
AEMS 100	4/6	53.25 ± 5.85	276.5 ± 169.52**	5/6	33.33	83.33

Results are expressed as mean ± SEM (n = 6), One-way ANOVA followed by Dunnett's post hoc test, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ *vs.* control; NC# = No convulsions.

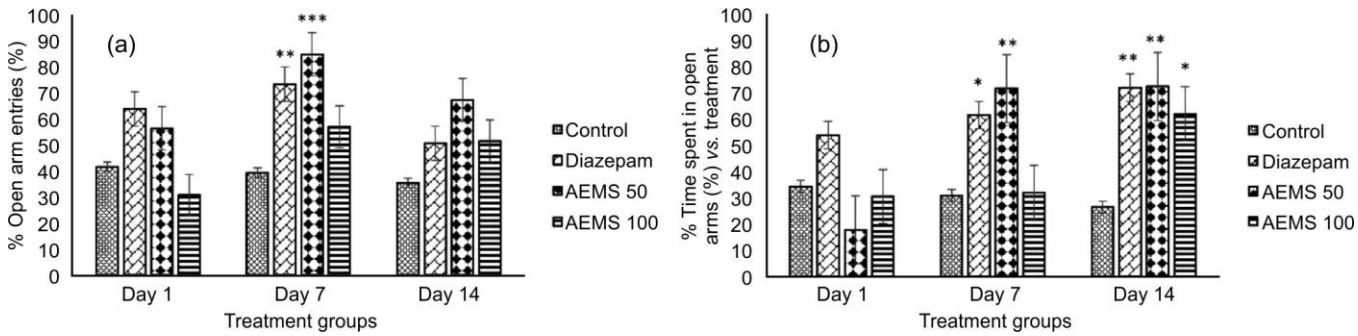

Fig. 2. Effects of AEMS in Elevated Plus Maze test. Results are expressed as mean ± SEM (n = 6), One-way ANOVA followed by Dunnett's post hoc test, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ *vs.* control

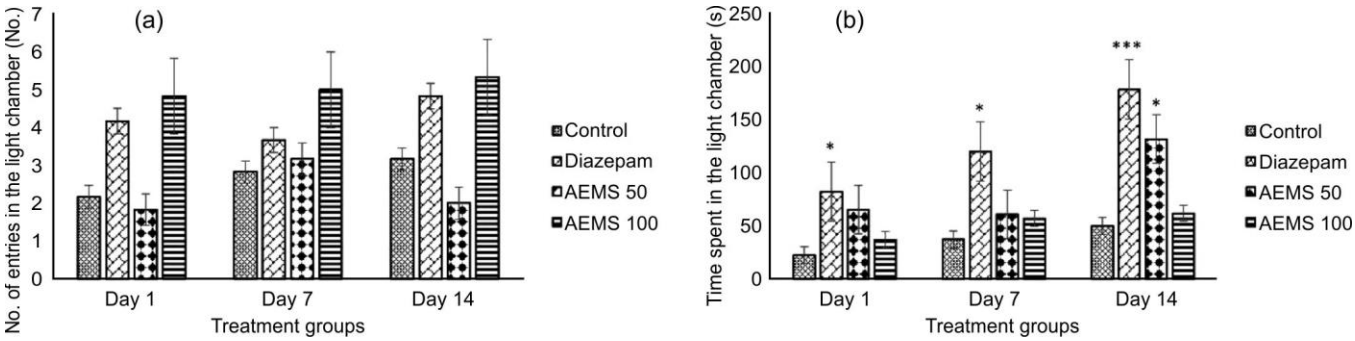

Fig. 3. Effects of AEMS in the Light and Dark model. Results are expressed as mean ± SEM (n = 6), One-way ANOVA followed by Dunnett's post hoc test, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ *vs.* control

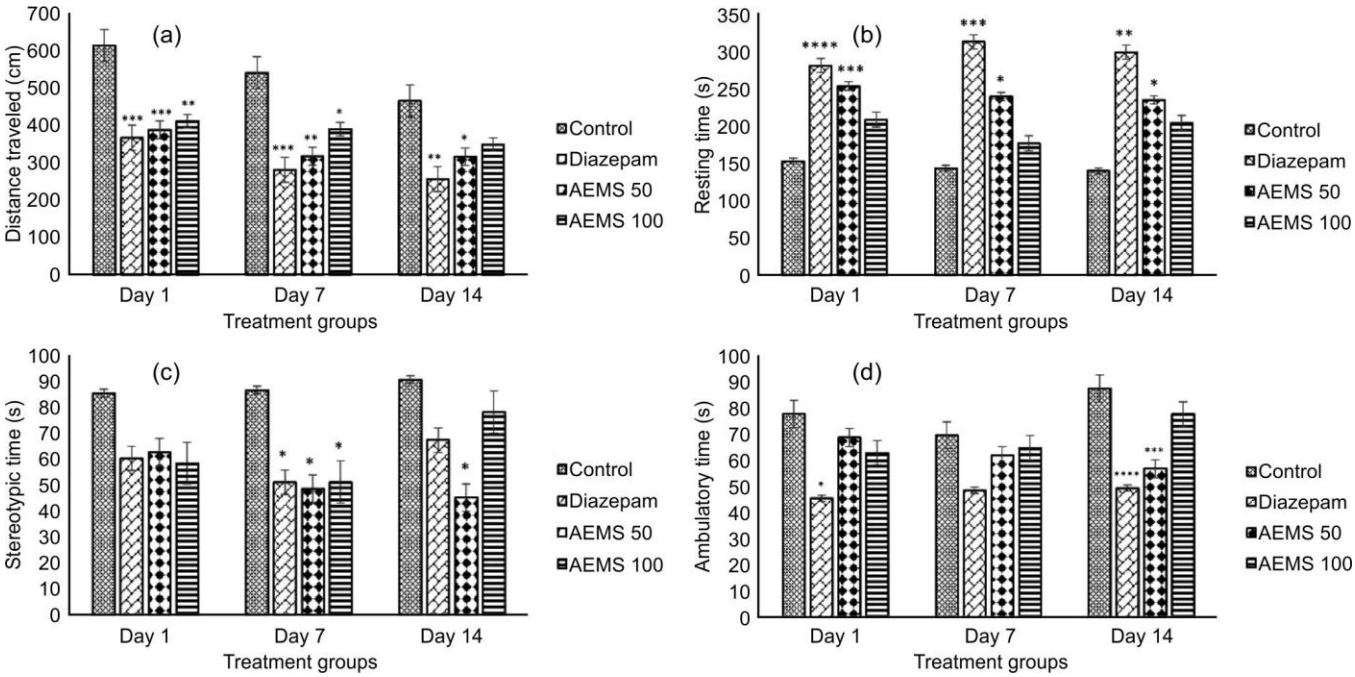

Fig. 4. Effects of AEMS in the Open Field test. Results are expressed as mean ± SEM (n = 6), One-way ANOVA followed by Dunnett's post hoc test, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ *vs.* control

moderate doses and diminishes at higher doses. Similar dose-response profiles have been reported for other anxiolytic agents; for example, Guimarães *et al.* [33] reported that cannabidiol (CBD) exhibited an inverted U-shaped dose-response curve, with intermediate doses producing greater anxiolytic effects than both lower and higher doses.

***In silico* docking simulation studies:** The molecular docking results for the phytoconstituents identified in AEMS, together with the reference ligand diazepam, against the $GABA_A$-benzodiazepine receptor (PDB ID: 6X3X) are shown in Table-4. Docking simulations predicted favourable interactions of the identified alkaloids with the $GABA_A$-benzodiazepine binding site, with binding affinities approaching that of diazepam. Diazepam exhibited a binding energy of -8.8 kcal/mol at the $GABA_A$-BZD-Cl^- ion channel receptor, whereas mitragynine and 7-hydroxymitragynine showed binding energies of -7.1 and -7.7 kcal/mol, respectively. Both alkaloids interacted with key amino acid residues including MET236, PRO233, PHE289 and MET286, producing interaction profiles comparable to those of diazepam. Corynoxine B also exhibited

TABLE-4
In silico MOLECULAR DOCKING OF THE IDENTIFIED COMPOUNDS FROM AEMS AND DIAZEPAM WITH $GABA_A$-BZD RECEPTOR (PDB ID: 6X3X)

Ligands	PubChem CID	Interacting Residues	Binding affinity (kcal/mol)
Diazepam (standard)	3016	LEU285, PHE289, MET286, LEU232, PRO233, MET236	-8.8
Mitragynine	3034396	MET236, VAL290, PHE289, LEU240, THR265, VAL258, LEU259, PRO233	-7.1
7-Hydroxymitragynine	44301524	MET236, PRO233, LEU269, THR266, ASN265, ILE228	-7.7
Corynoxine B	10091424	MET286, ILE228, GLN229, ASN265, THR266, PHE289	-6.6

appreciable binding affinity with a docking score of -6.6 kcal/mol (Fig. 5). The interaction of these alkaloids with the benzodiazepine binding site supports their potential role in modulating GABAergic neurotransmission, thereby enhancing inhibitory signalling associated with seizure suppression and anxiolytic activity. These findings provide mechanistic support for the central effects of AEMS observed in the *in vivo* studies [34].

Similar observations have been reported for other plant-derived anticonvulsant and anxiolytic agents. Erysothrine, an alkaloid isolated from the flowers of *Erythrina mulungu* Mart. ex Benth., exhibited anticonvulsant and anxiolytic effects in male wistar rats [35]. Talom *et al.* [34] reported that ethanolic and aqueous extracts of *Lantana camara* significantly attenuated seizures and anxiety-like behaviour in a kainic acid-induced mouse model, with evidence supporting GABAergic involvement. The dual anticonvulsant and anxiolytic profile observed with AEMS also resembles the pharmacological actions of benzodiazepines, which are clinically employed for the management of both conditions [35].

Conclusion

The present study demonstrates that the alkaloid-enriched fraction of *Mitragyna speciosa* (AEMS) leaves possesses significant anticonvulsant and anxiolytic activity in experimental animal models. AEMS increased seizure resistance and atten-

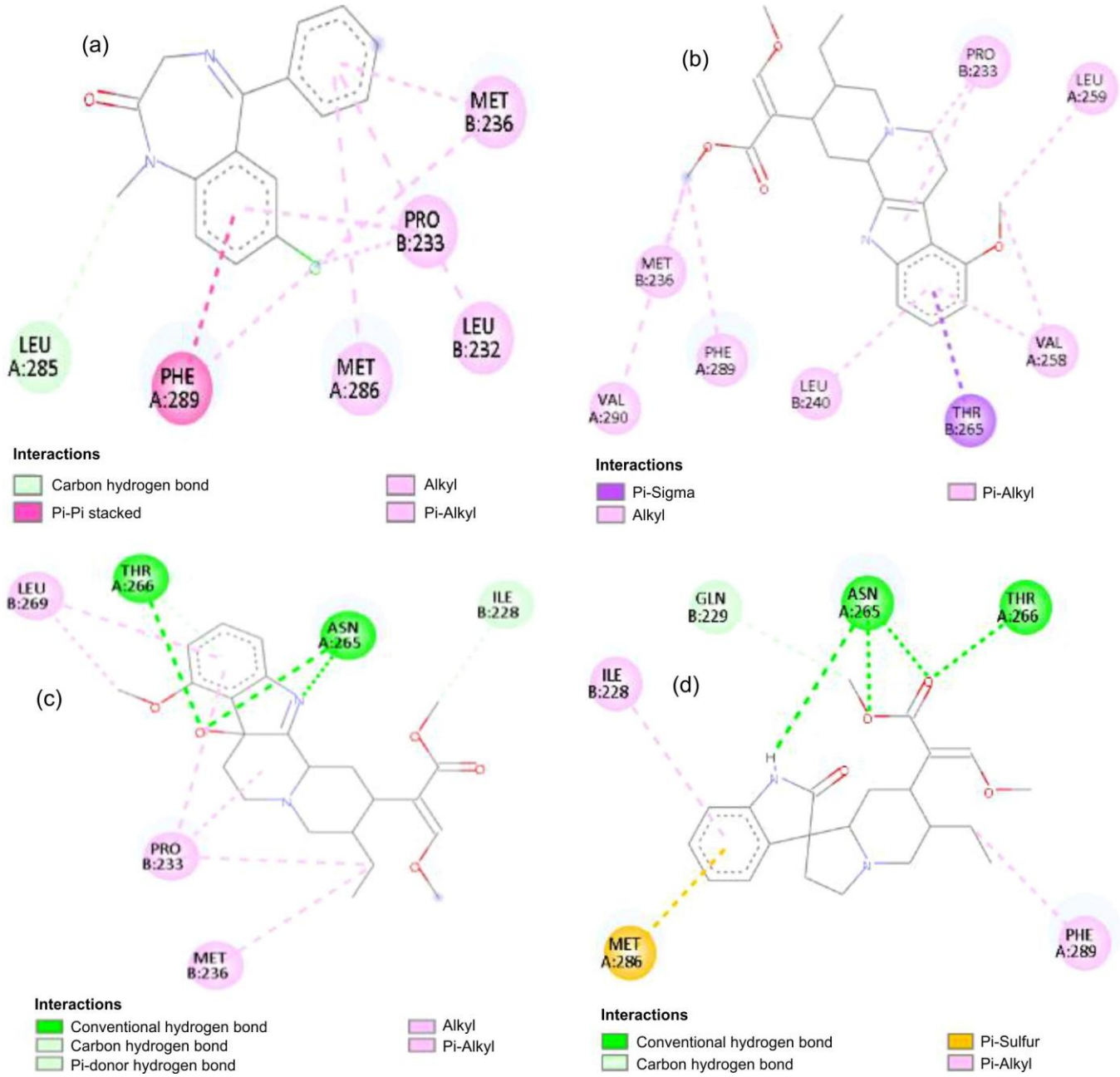

Fig. 5. Binding interactions of (a) diazepam, (b) mitragynine, (c) 7-hydroxymitragynine, (d) corynoxine B with $GABA_A$-BZD receptor (PDB ID: 6X3X)

uated anxiety-like behaviours, with molecular docking studies supporting modulation of the $GABA_A$-benzodiazepine receptor complex as a plausible underlying mechanism. These findings suggest that *M. speciosa* alkaloids exhibit neuropharmacological properties extending beyond their established opioid related actions. The superior efficacy observed at the lower dose highlights the importance of dose optimisation and warrants further mechanistic investigation. Future studies should focus on the isolation and characterization of the active constituents, evaluation of long-term efficacy and safety and assessment of their translational potential for the management of epilepsy and anxiety disorders.

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CONFLICT OF INTEREST

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

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language and no data, results or scientific conclusions were generated by AI. All analyses, interpretations and conclusions are the authors' own. The authors reviewed and edited the content and take full responsibility for the published work.

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