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FULL PAPER

# Maceration, phytochemical screening, and in vivo neuro-pharmacological evaluations of Rynchotechum Ellipticum acetone fraction in Swiss albino mice

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*Rhynchotechum ellipticum*, locally known as Cheodhima, Paicha, or Sattari, is a traditional medicinal plant used to treat various diseases. This study aimed to evaluate the neuropharmacological effects of R. ellipticum leaf and stem extracts in Swiss albino mice. In the thiopental sodium-induced sleeping time test, mice received different doses of 25, 50, 100, and 200 mg/kg body weight, while 50 and 125 mg/kg body weight were used in other examinations. In trials examining the effects of thiopental sodium on sleep, administration of the extracts caused a marked reduction in the amount of time spent sleeping and the time needed to fall asleep ($p$<0.05, $p$<0.01, and $p$<0.001). Additionally, at doses of 50 and 125 mg/kg, a significant decrease in unrestricted movement and exploratory behavior was observed in both the hole-crossing and open-field tests ($p$<0.05, $p$<0.01, and $p$<0.001). The hole-board test also decreased the frequency of head dips at doses of 50 mg/kg and 125 mg/kg ($p$<0.05, $p$<0.01, and $p$<0.001). Both extracts demonstrated superior neuropharmacological properties at a dose of 125 mL/kg. The leaf and stem extracts of *R. ellipticum* have potent anti-depressant and hypnotic effects on the central nervous system, and could be utilized in neuropharmacology as an additional therapy.

**KEYWORDS**

*Rynchotechum ellipticum*; neuropharmacological; *in vivo*; sleeping duration test; mice; extract.

## Introduction

The common and persistent psychiatric condition of depression seriously impairs social interaction and work productivity. Vast communities of different age groups currently suffer from this disease. According to data from the World Health Organization (WHO), depression is expected to rank as the second most common cause of disability worldwide by 2020. Furthermore, until 2030, depression is predicted to have the most significant effect

on the prevalence of diseases [1]. In Central Nervous System (CNS), depression is a severe disorder characterized by mood alterations, disinterest in the environment, apathy, decreased energy, psychomotor retardation, and pervasive emotions of despondency, hopelessness, and thoughts of committing suicide [2]. Avoiding activities that affect a person's thoughts, attitudes, feelings, and sense of well-being is a hallmark of depression, which is a mood illness. People who are depressed frequently feel lonely, anxious, worried, pessimistic, powerless, unproductive, regretful, angry, embarrassed, or irritated [3]. They were not motivated to engage in once-reward activities, hunger, or overeat. Physical changes also occur in severe, vital, or melancholic sadness [4].

Major depressive illnesses and other mental disorders may be manifested as mood depression. However, it can also be a rational response to life events, such as bereavement, signs of a few physical illnesses, or side effects of a few medications and medical procedures. In patients with severe depression, changes in monoamine neurotransmitters in the brain, particularly norepinephrine, serotonin, and dopamine, can result in symptoms [5,6]. Psychiatric depression, a subtype of major depression [7]. Acute psychosis may present a diagnostic obstacle. Several issues may be involved, including potentially fatal ones [8]. Because they induce sleepiness and manage sleep duration, sedatives and hypnotics have calming effects and are used to treat anxiety [9]. Significant public health hazards are associated with insomnia, a sleep problem that is becoming more widespread in society. Consequently, several researchers are studying sleep-inducing substances of different chemical types and exploring alternative therapies to address this issue [10]. Benzodiazepines are the most popular and widely used hypnotics.

Recently, they have been phased out in favor of modern non-benzodiazepine hypnotic therapies, including melatonin [11]. Selective serotonin reuptake inhibitors (SSRIs) are a family of antidepressants frequently administered to patients. An imbalance in serotonin levels has also been linked to depression. SSRI medications treat depressive symptoms, thereby declining serotonin reuptake in the brain [12]. Serotonin and noradrenaline reuptake is inhibited by tricyclic antidepressants (TCAs). Monoamine oxidase inhibitors (MAOIs) inhibit the monoamine oxidase enzyme, which breaks down dopamine, serotonin, and norepinephrine in the brain and other body parts [13]. However, they have several serious side effects, including sleeplessness, anxiety, insomnia, arrhythmia (irregular heartbeat), rash, weight loss, abdominal cramps, constipation, sweating, drowsiness, tremors, nausea, vomiting, seizures, and insomnia [14].

Plant-based alternative medicine plays a significant role in the health care systems of both developed and developing countries. It has been widely documented throughout history and worldwide that medicinal plants can treat various diseases. However, there has been a noticeable increase in the use of natural therapies [15]. Plant-based herbal medicines and nutritional supplements are becoming increasingly popular globally because they are affordable and less hazardous than synthetic drugs. The manufacturing of synthetic medications made from natural sources has significantly increased recently, although herbal medicines may not be widely available for commercial use. The therapeutic management of several illnesses, including neurological disorders such as Alzheimer's disease, Parkinson's disease, and others, however, significantly benefit from the secondary metabolites included in herbal therapies. In recent decades, natural products are of high himportance as a source of medicinal agents. Depending on their traditional use, several modern synthetic drugs have been developed from naturally occurring ingredients [16].

Chaudhuri *et al.* (2018) reported a comprehensive nutraceutical evaluation of R. ellipticum leaves, demonstrating high levels of total phenolics (617.30 ± 3.31 mg GAE/100 g) and flavonoids (265.93 ± 0.82 mg/100 g), and reporting strong *in vitro* antioxidant activity—attributes often linked to neuroprotective effects [17]. Additionally, R. ellipticum is a highly nutritious wild edible plant consumed by tribal communities in Northeast India, underlining its traditional dietary and medicinal use as supporting evidence for its potential health benefits [17]. Cheodhima, Paicha, and Sattari are all names for the herbal remedy *Rhynchotechum ellipticum*. It belongs to the family Gesneriaceae and is a vertical undershrub, with the heaviest stems reaching heights of 0.6-1.2. The reverse leaves were at least 16.5 cm long, broadly elliptical or obovate, minutely dentate, base cuneate, and white underneath. The berry had a diameter of 6 mm and was surrounded by umbellate cymes with rose-purple blooms in the lower axils. It has previously been utilized to treat cough in children [18]. Akhtar (2022) reported the traditional use of R. ellipticum in regions such as Sylhet and Chittagong for treating ailments such as cough and neurological symptoms, supporting its historical CNS-related applications [18]. This study investigated the sedative, anxiolytic, and antidepressant effects of *R. ellipticum* leaf and stem extracts in mice, a topic that has not been previously explored in experimental research.

## Experimental

### *Chemicals and reagents*

Thiopental sodium and diazepam were purchased from Clintech Research Solutions (Hyderabad, India). All other chemicals and reagents were purchased from Lab Trading Laboratory (Aurangabad, Maharashtra, India).

### *Plant materials*

The *Rhynchotechum ellipticum* were collected from Meghalaya state, India in December, 2023 and identification was authenticated by Sri Venkateswara University, Tirupati, India with voucher number 0661.

### *Drying and grinding*

The leaves and stems of *R. ellipticum* were washed with distilled water, dried, and processed into coarse powder using a proper processor. The leaves and stems were dried in secrecy to protect the active ingredients from oxidation and to maintain a safe distance from photochemical degradation. A proper processor was employed to ground the leaves and stems into a coarse powder. Before starting the investigation, the powder was carefully sealed inside a container and kept in space with minimal moisture, light, and temperature fluctuations.

### *Preparation of acetone extracts*

Granulated leaves and stems (300 g and 350 g) of *R. ellipticum* were soaked for ten days in separate glass compartments containing 1400 mL and 1800 mL of acetone, respectively. The mixture was then rigorously filtered through a small amount of fine white cotton. They were subsequently separated using Whatman No. 1 filter paper. Filtration was set up in an airy space for the solvent to evaporate, and the extracts were collected. The yield values of the acetone extracts from the leaves and stems were 2.07% weight per weight and 2.11% weight per weight, respectively. Acetone was selected as the solvent due to its intermediate polarity and its ability to efficiently extract a wide range of phytoconstituents. It also evaporates quickly and helps to preserve thermolabile compounds during extraction.

*Experimental animals*

Swiss albino mice (22-25 g, aged 6-7 weeks) were maintained in animal cages under typical natural conditions (22-25 °C, moisture 60-70%, and 12-hour light: 12-hour dull cycle). A typical pellet diet was provided to mice. The Research Ethics Committee of the Systemic Life Sciences and Research Private Limited, Hyderabad, Telangana, India, authorized the present research and *in vivo* studies (Ethical Approval No. 23/IAEC/SLSRPL/2024).

*Qualitative phytochemical screening*

Phytochemical analysis of *R. ellipticum* leaf and stem extracts was performed using the method described by Patra *et al.* [19] to identify secondary metabolites, such as tannins, phenols, flavonoids, saponins, terpenoids, gum, alkaloids, and glucosides, using a signature of color alteration.

*Quantitative determination of phytochemical constituents*

Alkaloids were examined using Harborne's (1973) technique. The item was carefully weighed to make an amount of 5 g, and then placed in a beaker with a capacity of 250 mL. The beaker was subsequently filled with 200 mL of an acetic acid solution containing 10% acetic acid in ethanol. Afterward, the beaker was sealed and unattended for four hours. Following the solution filtration, both extracts were diluted to 25% of their original volume using a water bath. Drops of concentrated ammonium hydroxide were gradually added to both the extracts until a precipitate was observed. Before filtering, the entire solution was allowed to sediment, and then rinsed with diluted ammonium hydroxide solution. The amount of alkaloid remaining after drying was also measured. The method developed by Van-Burden and Robinson (1981) was employed to determine tannin concentration. A plastic container with a capacity of 50 mL and a sample weighing 500 mg were used. A mechanical shaker containing 50 mL distilled water was agitated for 1 h. The solution was then carefully poured into a volumetric flask with 50 mL capacity and adjusted to the desired concentration. The filtrate was subsequently added to a test tube containing 2 mL of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. Absorbance was measured at a wavelength of 120 nm. The procedure for determining glycosides, as suggested by Bohm and Kocipai Abyazan in 1994, was as follows: a plant sample weighing 10 g was exposed to repeated extractions at ambient temperature using 100 ml of 80% aqueous methanol. The solution (125 mm) was filtered using Whatman filter paper no. 42. The filtrate was placed in a beaker and dried in a water bath until a consistent weight was obtained [20-22].

*Thiopental sodium-induced sleeping time test*

The procedures outlined were used in the study by Ali *et al.* (2015) [23] to examine the effects of the two extracts on sleeping time with thiopental sodium. For this scenario, the mice were separated into ten groups, of five mice each. The control group was administered an oral dose of distilled water. The standard was orally administered to Group II and was diazepam (0.5 mg/kg, b.w., p.o.). Leaf extracts were orally administered to Groups III, IV, V, and VI at 25, 50, 125, and 200 mg/kg body weight. Oral stem extract was administered to experimental groups VII, VIII, IX, and X at doses of 25, 50, 125, and 200 mg/kg body weight. All experimental groups received an intra-peritoneal dose of thiopental sodium (20 mg/kg b.w.) to put them to sleep after thirty minutes. Individual mice were placed on a table and their erratic movements were recorded. The following formula was used to determine the proportion of the effect:

$$\text{Effect (\%)} = \frac{\text{Average duration of loss of righting reflex in the test group}}{\text{Average duration of loss of righting reflex in the control group}} \times 100 \quad (1)$$

*Hole cross test*

As mentioned previously, this study was conducted by Uddin *et al.* (2006) [24]. A cage with the dimensions 0.30 ×0.20 ×0.14 m was used. The center of the enclosure was fastened to a partition. At a vertical point of 0.075 m, precisely in the center of the frame, a circular aperture with a diameter of 0.03 m was created. Three groups (control, standard, and extract) were formed from the animals. Subsequently, they are positioned on one side of the frame. Quantification of mouse passes through the hole was carried out for 3 min at 0, 30, 60, 90, and 120 min after delivering the control, standard, leaf, and stem extracts from one chamber to the next. Six different classes of mice were utilized in Group I, the test group, and distilled water was orally administered. Group II received oral diazepam (1 mg/kg, p.o.) as the benchmark for comparison. In groups III and IV, the leaf extracts were administered at 50 and 125 mg/kg body weight, respectively. Stem extracts were then administered orally to groups V and VI at 50 mg/kg and 125 mg/kg body weight, respectively.

$$\text{Movements Inhibition (\%)} = \frac{\text{Mean No.of movements (control)} - \text{Mean No.of movements (test)}}{\text{Mean No.of movements (control)}} \times 10 \quad (2)$$

*Hole board test*

This study followed the methods described by Sheikh *et al.* [25] with a few minor adjustments. The level base used in this experiment was 0.90 m × 0.90 m in radius, with 16 holes placed equally apart. Additionally, this particular stage had a 0.05-meter-tall frame installed. Six groups of mice control, reference, and test were created. Five mice (n=5) were used in each group. Oral distilled water was administered to Group 1 under the control scenario. Diazepam was administered orally to group II at a dose of 1 mg/kg body weight. The leaf and stem extracts were administered orally to Groups III, IV, and V at doses of 50 and 125 mg/kg body weight, respectively. Over ten minutes, we observed how often each mouse's head doved into holes.

$$\text{Inhibition (\%)} = \frac{\text{Mean No.of head dips (control)} - \text{Mean No.of head dips (test)}}{\text{Mean No.of head dips (control)}} \times 100 \quad (3)$$

*Open field test*

This analysis was performed as previously described by Anisuzzman *et al.* [26]. A planar field with a square pattern of size 0.5 square meters in size makes up the experimental setup. On the other hand, the squares on the opposing side are painted in alternate rows of black and white. The experimental board had chessboard-like characteristics. A compartment height of 0.1 meters was also present in the mechanical system. Six groups of mice were used for this study. Five mice (n=5) were divided into two groups. Distilled water was orally administered to the control group. Group II received diazepam (1 mg/kg, bw, p. o.) orally as the standard. The 50 and 125 mg/kg body weight fractions of leaf and stem extracts were administered orally to Groups III, IV, and V, respectively. The number of squares the animals moved at any speed for three minutes starting at 0, 30, 60, 90, and 120 min after oral administration of the test drugs.

$$\text{Movements Inhibition (\%)} = \frac{\text{Mean No.of movements (control)} - \text{Mean No.of movements (test)}}{\text{Mean No.of movements (control)}} \times 100 \quad (4)$$

*Statistical analysis*

The data were examined using SPSS statistical software (version 20). The findings were expressed as the mean ± standard deviation (SD) and mean±SEM. To enable comparisons across groups, one-way ANOVA with Dunnett's tests for sleeping time, hole board, hole cross, and open field tests were employed.

## Results and discussion

Traditional medicinal plants have been utilized for their therapeutic properties since prehistoric times, and their products are frequently used in the pharmaceutical, nutraceutical, and food supplement sectors for herbal medications, minerals, and dietary supplements [27]. The side effects of CNS medications, including anticholinergic effects, abuse, and cognitive dysfunction, can affect patient adherence and tolerance, potentially leading to acute withdrawal symptoms upon discontinuation [28,29]. Consequently, it is essential to develop novel therapeutic drugs with improved tolerability and increased efficacy to optimize the treatment of depressive and phobic illnesses. The sedative effects of the leaf and stem extracts of R. ellipticum were assessed by observing and recording the typical locomotor behavior of mice in hole-cross and open-field studies, the sedative effects of leaves and stem extracts of *R. ellipticum* were assessed. These results suggest that the parameter under consideration can be utilized as a predictor for evaluating the motor function of an animal model after administration of leaf and stem extracts. The outcomes were compared between the standard and control groups. Any substance with sedative properties in these studies would decrease the frequency and intensity of inferred gestures, indicating a decline in interest in a new environment.

*Qualitative phytochemical screening*

Both extracts contained major groups, such as tannins, phenols, flavonoids, saponins, terpenoids, gum, glycosides, and alkaloids, as indicated in Table 1 from the phytochemical analysis of the samples. Phenols, saponins, and gums were absent in the leaf extract, while flavonoids were absent in the stem extract.

The maceration technique was selected for extraction due to its effectiveness in preserving thermolabile phytoconstituents and capability to extract a wide range of bioactive compounds with minimal degradation. This traditional solvent-based method ensures that the chemical integrity of the plant remains largely intact, making it suitable for preliminary phytochemical screening.

**TABLE 1** Phytochemical screening of both extracts

| Tested groups | Leaf extract | Stem extract |
|---|---|---|
| **Tannins** | + | + |
| **Phenols** | - | + |
| **Flavonoids** | + | - |
| **Saponins** | - | + |
| **Terpenoids** | + | + |
| **Gum** | - | + |
| **Alkaloids** | + | + |
| **Glycosides** | + | + |

*Note*: +, present; -, absent.

*Quantitative determination of phytochemical constituents*

The quantitative estimation of key phytochemical groups, tannins, glycosides, and alkaloids, was conducted to assess the relative abundance of these bioactive compounds in both leaf and stem extracts of Rhynchotechum ellipticum. The results, summarized in Table 2, revealed that tannins were present at the highest concentration, particularly in the leaf extract (0.45 ± 0.22%), followed by glycosides (0.31 ± 0.12%) and alkaloids (0.22 ± 0.18%). In the stem extract, alkaloids (0.27 ± 0.07%) slightly exceeded tannins (0.29 ± 0.14%), while glycosides were the least concentrated (0.23 ± 0.11%). These variations in the phytochemical composition between the leaf and stem may contribute to their distinct pharmacological effects. Tannins are known for their antioxidant, antimicrobial, and CNS-modulating properties, while alkaloids and glycosides are associated with neuroactive, cardioprotective, and mood-stabilizing effects. Therefore, the quantitative results support the hypothesis that the observed neuropharmacological activities are partly due to the presence of these phytochemicals and their relative abundance in the extracts.

**TABLE 2** Percentage of tannins, glycosides, and alkaloids in the *Rhynchotechum ellipticum* extracts

| Phytochemical class | Quantity (%) | |
|---|---|---|
| | Leaf | Stem |
| **Tannins** | 0.45±0.22 | 0.29±0.14 |
| **Glycosides** | 0.31±0.12 | 0.23±0.11 |
| **Alkaloids** | 0.22±0.18 | 0.27±0.07 |

The values are represented as Mean± SD.

*Sleeping time test*

Hypnosis was induced as a part of the experiment using thiopental sodium. The time taken for sleep to start was significantly shortened after administration of both extracts at several doses (25, 50, 100, and 200 mg/kg). Additionally, there was a significant dose-dependent improvement in the duration of sleep. This difference was statistically significant ($p < 0.001$). All dosages of the extract increased the duration of the thiopental sodium-induced sleep phase in mice compared to that in the controls. In this investigation, diazepam (0.5 mg/kg) exhibited a 529.29% effect. In contrast, the leaf and stem extracts at 200 mg/kg had peak effects of 604.81% and 585.56%, respectively, during corrective reflex failure (Table 3).

**TABLE 3** Sleeping time in mice was induced by the effect of *Rhynchotechum ellipticum* extracts on thiopental-Na

| Group | Dose(mg/kg) | Latent period | Sleeping time | % Effect |
|---|---|---|---|---|
| **Control** | 10 ml/kg | 9.6±0.92 | 37.4±1.4 | 0 |
| **Standard** | 0.50 | 2.6±0.24 | 198.2±4.71*** | 529.95 |
| **Leaf Extract** | 25 | 9.3±0.48 | 35.8±1.80 | 95.72 |
| **Leaf Extract** | 50 | 5.8±0.37 | 77.2±3.07** | 206.42 |
| **Leaf Extract** | 100 | 3.9±0.33 | 142.0±3.69*** | 379.68 |
| **Leaf Extract** | 200 | 2.1±0.18 | 226.2±14.84*** | 604.81 |
| **Stem Extract** | 25 | 9.4±0.50 | 31.8±2.08 | 85.03 |
| **Stem Extract** | 50 | 6.0±0.54 | 87.0±6.44*** | 232.62 |
| **Stem Extract** | 100 | 4.1±0.33 | 148.0±9.43*** | 395.72 |
| **Stem Extract** | 200 | 2.2±0.25 | 219.0±11.01*** | 585.56 |

Values are presented as mean ± SEM (n = 5). The significance levels are indicated as *$p$<0.05, **$p$<0.01, and ***$p$<0.001, demonstrating statistical significance compared with the control group. One-way ANOVA followed by Dunnett's test was employed for statistical analysis.

*Hole cross test*

The mice in the control group varied in the number of holes they traversed from one chamber to another during the hole crossing test from 30 to 120 min. The leaf and stem extracts exhibited a continual drop in activity in laboratory mice at doses of 50 and 125 mg/kg from the second to the last analyses. Pertinent effects ($p$ < 0.001) were dose dependent (Table 4).

**TABLE 4** Neuropharmacological potential test of leaves and stems extracts of *Rhynchotechum ellipticum* using hole cross method

| Group | Dose (ml/kg) | Number of movements (% of movements inhibition) | | | | |
|---|---|---|---|---|---|---|
| | | 0 min | 30 min | 60 min | 90 min | 120 min |
| **Control** | 10 | 5.0±0.70 | 6.2±0.37 | 6.2±0.48 | 6.6±0.40 | 8.6±0.24 |
| **Standard** | 1 | 1.6±0.50 | 1.2±0.37*** | 1.6±0.48*** | 1.2±0.58*** | 0.2±0.2*** |
| **Leaf Extract** | 50 | 1.0±0.31 | 1.6±0.4*** | 1.0±0.54*** | 1.6±0.50*** | 1.4±0.50*** |
| **Leaf Extract** | 125 | 0.6±0.4 | 0.8±0.37*** | 1.0±0.31*** | 1.4±0.5*** | 1.4±0.50*** |
| **Stem Extract** | 50 | 2.8±0.86 | 2.0±0.44*** | 1.6±0.40*** | 2.2±0.37*** | 2.0±0.63*** |
| **Stem Extract** | 125 | 1.6±0.4 | 1.8±0.37*** | 1.8±0.73*** | 1.6±0.24*** | 1.6±0.24*** |

Values are presented as mean ± SEM (n = 5). The significance levels are indicated as *$p$<0.05, **$p$<0.01, and ***$p$<0.001, demonstrating statistical significance compared to the control group. One-way ANOVA followed by Dunnett's test was used for statistical analysis.

*Hole board test*

At the fifth observation point in this test, the combined effect of the leaf and stem extracts at 125 mg/kg resulted in 75.71% and 73.84% inhibition of locomotor activity. Research on diazepam has revealed a 97% suppression of action. Evaluation of the effects of leaf and stem extracts on the central nervous system produced exciting results because the dosage significantly affected the animals' ability to perform the hole-board test without experiencing head dips. The movement of stem extracts was 73.84% more effectively suppressed by 125 mg/kg diazepam than by the stem extracts (Table 5).

**TABLE 5** Neuropharmacological insights of *Rhynchotechum ellipticum* leaves and stem extracts assessed utilizing the hole board method.

| Group | Dose(mg/kg) | Number of head dips | % inhibition |
|---|---|---|---|
| **Control** | 10ml/kg | 21.4 ±2.97 | 0 |
| **Standard** | 1 | 7±0.70 | 67.29*** |
| **Leaf Extract** | 50 | 6.4±0.4 | 70.09*** |
| **Leaf Extract** | 125 | 5.2±0.58 | 75.71*** |
| **Stem Extract** | 50 | 7.4±0.87 | 65.43*** |
| **Stem Extract** | 125 | 5.6±0.81 | 73.84*** |

Values are presented as mean ± SEM (n = 5). The significance levels are indicated as *$p$<0.05, **$p$<0.01, and ***$p$<0.001, demonstrating statistical significance compared to the control group. One-way ANOVA followed by Dunnett's test was used for statistical analysis.

*Open field test*

All test extracts significantly reduced locomotor function in mice at doses of 50 and 125 mg/kg body weight ($p$ < 0.05, $p$ < 0.01, $p$ < 0.001, respectively), and this effect was present from the first study period and remained until the fifth study period (Table 6). Diazepam (1 mg/kg) significantly reduced the ability of mice to move from the second to fifth research period. In this test, the most significant locomotor activity suppression achieved by the stem extract at 125 mg/kg was 45.2%, compared to 47.4% suppression achieved by diazepam.

**.TABLE 6** Neuropharmacological potential test using the open field method *Rhynchotechum ellipticum* leaf and stem extracts

| Group | Dose | Number of movements (% of movements inhibition) | | | | |
|---|---|---|---|---|---|---|
| | | 0 min | 30 min | 60 min | 90 min | 120 min |
| **Control** | 10 ml/kg | 26.8±1.56 | 28.0±2.38 | 28.6±2.15 | 31.8±2.13 | 37.6±2.01 |
| **Standard** | 1 | 14.2±3.33 | 14.8±3.91** | 16.4±3.26*** | 18±3.11*** | 19.8±3.48*** |
| **Leaf Extract** | 50 | 18.0±2.28 | 20.0±1.30 | 19.0±0.94** | 19.6±1.16*** | 20.0±0.44*** |
| **Leaf Extract** | 125 | 15.8±1.46 | 17.4±2.20* | 17.2±1.2*** | 18.4±1.4*** | 21.6±1.56*** |
| **Stem Extract** | 50 | 17.4±0.92 | 19.4±1.12* | 19.2±1.11** | 20.4±1.4** | 20.2±1.15*** |
| **Stem Extract** | 125 | 16.2±1.01 | 17±1.22** | 16±1.14*** | 17.8±1.28*** | 20.6±1.20*** |

Values are presented as mean ± SEM (n = 5). The significance levels are indicated as *$p$<0.05, **$p$<0.01, and ***$p$<0.001, demonstrating statistical significance compared to the control group. One-way ANOVA followed by Dunnett's test was employed for statistical analysis.

The findings showed that oral administration of both leaf and stem extracts at all dosages (50 and 125 mg/kg) significantly decreased ($p$ < 0.001) the number of crossed holes (Table 4). After administering the leaf and stem extracts for up to 120 min, the suppressive activity persisted for 30 min. The open field test is frequently recognized as the most reliable way to separate people based on their exploration rate. This test is frequently employed to evaluate the effects of mouse stimulants on locomotor activity [30]. Test samples at the doses in the open field test displayed significant locomotor inhibition ($p$ < 0.05, $p$ < 0.01, and $p$ < 0.001), which increased from 30 min to 120 min over the measurement time (Table 5). According to the results of the current investigation (Tables 4 and 6), leaf and stem extracts facilitated locomotor functions, supporting depressive effects in the CNS.

CNS depression was observed in the open-field and hole cross-test samples. As presented in Tables 4, 5, and 6, the locomotor activity gradually decreased throughout the observation period. Besides the head-dipping test on the hole board, sedative and anxiolytic effects have been evaluated [31]. The head-dip test is a method for confirming anxiolytic-like effects [32]. Head dipping is frequently seen as a symbol of curious behavior. The results of these tests revealed that both the extracts had anxiolytic and anti-depressant effects. Hole-cross and open-field tests were employed to investigate locomotor inhibitory activity in mice [33,34]. Additionally, during the sodium thiopental-induced sleep time test, the leaf and stem extracts significantly decreased latency time and increased the length of time spent asleep, suggesting a potential sedative effect (Table 3). Both extracts exerted sedative and CNS depressive effects by increasing hyperpolarization-induced γ-aminobutyric acid-ergic (GABAergic) inhibition. This is accomplished by allosterically modulating GABA receptors, which shortens the time it takes to fall asleep and lengthens sleep duration [35-37]. Therefore, a potential modulatory effect on GABA receptors is suggested by the significant decrease in locomotor activity and the observed improvement in sleep patterns after extract administration. The initial phytochemical screening identified alkaloids, glycosides, saponins, flavonoids, and tannins. Research has revealed that plant substances rich in flavonoids, phenols, and tannins exhibit central nervous system (CNS) activity, including sedative, anxiolytic, and depressive effects [38,39]. Additionally, previous studies have revealed that alkaloids, flavonoids, and terpenoids have psychoactive qualities because they activate protein kinase C (PKC) and transcription factors that encourage the production of genes essential for cell survival.

This system protects the neurons from oxidative and metabolic damage. These substances also activate nicotinic receptors, thereby enhancing memory and cognitive functions. They also aid in restoration and enhancement of the functional capacity of the nervous system. Finally, they open calcium transient receptor potential channels in nerve cell membranes [40,41].

## Conclusion

The present study provides substantial experimental evidence supporting the neuropharmacological potential of Rhynchotechum ellipticum leaf and stem extract. *In vivo* testing in Swiss albino mice showed that both extracts exhibited significant sedative, anxiolytic, and CNS depressant activities, as evidenced by reductions in locomotor activity across the open field, hole cross, and hole board tests. The thiopental sodium-induced sleep test further confirmed the hypnotic efficacy of both extracts, with the highest effects observed at 200 mg/kg, comparable to or superior to the standard diazepam. Phytochemical screening revealed the presence of several bioactive groups, such as alkaloids, tannins, glycosides, terpenoids, and flavonoids, which are known to possess CNS-modulating properties. Quantitative analysis indicated that tannins and alkaloids are the predominant constituents, potentially contributing to the observed neuropharmacological effects. The significant activity observed suggests the involvement of GABAergic pathways, likely through the positive allosteric modulation of GABA-A receptors. The use of acetone as a solvent and the maceration technique proved effective in preserving thermolabile phytochemicals and ensuring a broad-spectrum extract. Overall, R. ellipticum is an optimal source of plant-derived neurotherapeutics that may serve as alternatives or complements to conventional CNS drugs, with fewer side effects. Further studies are necessary to isolate specific active compounds and elucidate their exact mechanisms of action through molecular and clinical investigations.

## Abbreviations

CNS: Central Nervous System
MAOIs: Monoamine Oxidase Inhibitors
GABA-A: Gamma-Aminobutyric Acid-A

## Ethical Approval

The Research Ethics Committee of the Systemic Life Sciences and Research Private Limited, Hyderabad, Telangana, India, authorized the present research and *in vivo* studies (Ethical Approval No. 23/IAEC/SLSRPL/2024).

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## Authors' Contributions

All authors have contributed equally to this work.

## Conflict of Interest

No conflict of interest was reported by the authors in this work.

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