

Extraction, Isolation and Neuropharmacological Potential of *Peristrophe bicalyculata* (R.) Nees

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ABSTRACT

Because of their widespread availability, low cost, low risk, and lack of side effects, medicinal herbs play a crucial role in traditional medicine all throughout the world. For medicinal purposes, *Peristrophe bicalyculata* (R.) is being used today. Nees as a nerve system tonic. Plant roots have antibacterial, anti-inflammatory, immunostimulating, and aphrodisiac properties. This plant root may provide health advantages, making it suitable for nutraceutical and functional food compositions. This research examined *P. bicalyculata* (R.) neuropharmacological effects on memory enhancement to treat cognition-related illnesses and learning and memory disorders. In phytochemical investigation, *P. bicalyculata* (R.) Nees ethanolic extract has higher phytoconstituents. Spectrophotometric and thin layer chromatography (TLC) plate bioassay detection showed that *P. bicalyculata* (R.) Nees ethanolic extract inhibits acetylcholinesterase activity. TLC and high-performance thin layer chromatography (HPTLC) were performed on *P. bicalyculata* (R.) Nees ethanolic extract. TLC and HPTLC spots are well resolved, indicating active ingredients in the extract. Total phenolic, flavonoid, and ascorbic content of *P. bicalyculata* (R.) Nees ethanolic extract was measured. *Ex-vivo* tests like lipid peroxidation testing reveal the extract is antioxidant. In conclusion, the ethanolic extract of *P. bicalyculata* (R.) Nees has memory-enhancing, antidepressant, and neuroprotective properties due to its anticholinesterase potential, effect on neurotransmitters like dopamine and serotonin, and antioxidant profile. More research is needed on its safety, effectiveness, and mechanism of action for therapeutic use.

Keywords: Extraction, Isolation, Neuropharmacological potential, Medicinal plants, *Peristrophe bicalyculata* (R.) Nees.

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INTRODUCTION

Currently, neurological disorders such as anxiety, depression, and epilepsy have a substantial impact on society. Sleep disturbances are associated with many illnesses and may be categorized as an independent pathology, in addition to serving as a symptom of these pathologies. Moreover, epilepsy is a grave neurological disorder that impacts 6.38 individuals per 1000 persons worldwide. Recent study indicates that over 322 million persons globally, which accounts for nearly 4.4% of the population, experience depression. The effectiveness of existing treatments for these illnesses varies depending on the individual, perhaps leading to treatment resistance in some situations. Throughout history, people have used several methods and procedures to address mental diseases, with medicinal plants often serving as a valuable resource. Medicinal plants provide a plethora of physiologically active chemicals that form the basis for pharmacological research and the identification of new compounds with hypnotic, anticonvulsant, anxiolytic, and antidepressant characteristics. Therefore, it is crucial to provide scientific evidence on the pharmacological characteristics of therapeutic plants and validating their uses.¹⁻³

Peristrophe bicalyculata Nees, often known as Retz's peristrophe, is a diminutive, pubescent, prostrate annual herb that reaches a height ranging from 20 to 90 cm. The object is vertical and compact. Mature shoots have a hexagonal shape and are covered in white, spreading bristle hairs. In contrast, juvenile shoots often have a quadrilateral shape. The leaves are acuminate, ovate, arranged in an opposing pattern, with equal or uneven sizes, measuring 2.5–7.5 x 2.0–4.0 cm. They are intact and have a greater amount of hair on the underside. The length of the petiole ranges from 6 to 12 mm. The plant produces pink flowers that are 1-cm in size and have two lips, with the bottom lip spreading outwards and the upper lip standing straight. These blooms grow at the terminals of branches or in the axils of leaves. The leaves of the plant create a large and loosely arranged panicle. Each of the two stamens has its own filament. A whitish object with a covering of fine hairs and a length of up to 5 mm is described. The number of smaller bracteoles is four, whereas the number of opposing, unequal, longer bracts relative to the calyx is two. The corolla is covered with fine hairs on the outside, giving it a pubescent appearance. It is pink in color and is roughly 12 mm in length.

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The capsules have an elliptical shape and taper into a pointed, cylindrical stalk measuring roughly 8 mm in length and 2 mm in width. They are also at the stage of puberty. The four seeds are compressed, and they are covered with little papillae. The process of fruiting and blooming occurs over the months of August to September. Petunidin-3-rhamnoglucoside is present in flowers, while volatile oil is present in roots. Sterols and fatty acids are found in both the stem and root. The stem methanolic extract contains free amino acids and sugars. Mucilage is found in the seeds, while alkaloids are present in the leaves and stems.

Experimentation was made to confirm the sedative action of a water soluble extract of Chaksini *P. bicalyculata* (R.) Nees on rats that were induced into a state of unconsciousness using pentobarbitone sodium 25 mg/100 g/body wt.dose. The result was deemed significant in terms of central depressive activity. Recent laboratory research indicates that the hydroalcoholic extract of *P. bicalyculata* (R.) Nees leaves showed reduced cytotoxicity and mutagenicity. Furthermore, this extract did not demonstrate any toxicity when administered to both male and female rats. Consequently, we performed a controlled study to ascertain if the hydroalcoholic extract derived from the leaves of *P. bicalyculata* (R.) Nees possesses psychopharmacological properties akin to other species within the same genus that are presently recognized as potent natural reservoirs and substitutes for the management of human ailments, particularly mental disorders.⁴⁻⁶ Previous research on the genus's neuropharmacological capabilities and safety led this.

MATERIAL AND METHODS

Plant and its Extraction Process

P. bicalyculata (R.) Nees stem bark was collected from a wooded area. After giving the stem bark a thorough cleaning, it was allowed to dry in the shade, mechanically crushed, and sieved using a No. 10/44 sieve (Figure 1). In a soxhlet apparatus, petroleum ether was used to defat the extract. The defatted material (600 g) was heated to 50°C for 16 to 17 cycles in an ethanol extractor and 1.5 L of chloroform for about 15 cycles in a hot extraction system to get the ethanol extract. 200 mL of water were boiled to 65°C for fifteen minutes with one hundred grams of plant material immersed in it. After that, the mixture was filtered to yield the water-based extract. The extracts were dried out in a vacuum. Desiccators were used to preserve the ethanolic and water-based *P. bicalyculata* (R.) Nees (PBE) extracts from the plant until additional research could be conducted.

Phytochemical Analysis

The phytochemicals included in the crude extracts are analyzed qualitatively. A study on *P. bicalyculata* (R.) Nees (PBE) revealed that the plant is a repository for many phytochemicals. The polar phytochemicals found in these extracts. These secondary metabolites have been researched and shown to have positive effects on health. There are other reports of phytochemicals found in the fruits and leaves of *P. bicalyculata* (R.) Nees. The current research clearly shows that *P. bicalyculata* (R.) Nees stem bark also has active groups



Figure 1: Stem bark of *P. bicalyculata* (R.) Nees

of phytochemicals that provide the plant its advantageous characteristics.^{7,8}

Pharmacological Test for Neuropharmacological Activity

Animals and ethics

We purchased female Swiss mice from the Central Animal Facility that weighed between 25 and 35 grams. The lights were turned on at 7:00 a.m. and the mice were kept in a 12:12 light/dark cycle. Because female mice are more accessible in the vivarium and had a greater frequency of sadness than male mice, this study employed female mice. To avoid any possible tampering with the results, only female participants with a regular estrous cycle were employed. To ensure the animals were not stressed, trained professionals performed behavioral tests in real time. The protocols adhered to the published recommendations for laboratory animals by the Indian government.⁹

Drugs and treatments

Sigma Chemical Co. supplied imipramine, sodium pentobarbital, strychnine, and pentylenetetrazol, along with other drugs used in the research. We obtained diazepam from Cristalia. The medicine was administered intraperitoneally (i.p.) at a dosage of 10 milliliters per kilogram of body weight, after its dissolution in 0.9% NaCl solution. preceding to administration, daily solutions of HEPC (50, 100, and 150 mg/kg) were prepared using distilled water and 2% DMSO. These solutions were then given orally. The negative control, referred to as the vehicle, was administered orally (p.o.) by gavage at a dosage of 10 mL/kg. The solution consisted of distilled water with 2% DMSO. An hour before to the trials, all HEPC and vehicle treatments were administered. Each experimental group consisted of 06–08 individuals, referred to as the experimental N.

Potentiation of diazepam-induced sleeping time

This examination uses animal models to assess the possessions of medications on the central nervous system (CNS). Many pharmacological investigations depend on barbiturates or other sedative drugs extending the length of sleep. The diazepam-induced prolongation of sleep duration was the foundation of the sleep evaluation method. The following categories apply to the groupings.

Six groups of animals were used in this study:

Group I (Control): received just a vehicle solution (2.5% tween 80) in order to take administration-related effects into consideration.

Group II (Diazepam): an intraperitoneal injection (i.p.) of diazepam at a dose of 2 mg/kg as a positive control for lowering anxiety.

Group III (Peristrophe + Diazepam 200 mg): received two doses of diazepam (i.p.) and a chloroform extract of *P. bicalyculata* leaves (20 mg/kg each) to evaluate any possible synergistic effects between the medication and the herb.

Group IV (Peristrophe + Diazepam 400 mg): received an oral dosage of 400 mg/kg of the chloroform extract and an intraperitoneal dose of 2 mg/kg of diazepam to investigate the extract's dose-dependent effects.

Group V (Peristrophe + Diazepam 200 mg ethanol): received 2 mg/kg of intraperitoneal (i.p.) diazepam and 200 mg/kg of an ethanol extract of *P. bicalyculata* leaves in order to examine the effects of various extraction techniques.

Group VI (Peristrophe + Diazepam 400 mg ethanol): received two doses of diazepam (i.p.) at a dose of 2 mg/kg and 400 mg/kg of the ethanol extract orally in order to compare the extraction process and dose-dependent effect at the same time.

The two parameters were assessed after the test drugs were injected. Durations of time between starting diazepam medication and righting reflex loss and between righting reflex loss and subsequent recovery are recorded. By comparing the incidence of early start and/or protracted sleep, the test medication's potential sedative and hypnotic action was found to be higher than that of the control group.

Spontaneous motor activity

The Digital Actophotometer was used to measure the spontaneous locomotor activity. This device operates on the basis that the instrument automatically detects the movement of experimental animals when there is an interruption of infrared beams. The evaluation of the CNS depressive or stimulating property was conducted using this approach (11). The groupings are categorized as:

Group I: 2.5 % tween 80 (Control group of animals received vehicle)

Group II: 2 mg/kg i.p (diazepam)

Group III: 200 mg/kg p.o. (Chloroform leaves extract of *P. bicalyculata* (R.) Nees)

Group IV: 400 mg/kg p.o. (Chloroform extract of leaves of *P. bicalyculata* (R.) Nees)

Group V: 200 mg/kg p.o. (Ethanol extract of leaves of *P. bicalyculata* (R.) Nees)

Group VI: 400 mg/kg p.o. (Ethanol extract of leaves of *P. bicalyculata* (R.) Nees)

Following the treatment, each rat's locomotor activity was automatically monitored for 120 minutes at 30-minute intervals.

Motor coordination

The Rotarod apparatus was used to assess the mice's motor coordination. Using this apparatus, researchers may look at how drugs affect motor coordination. During the course of the

experiment, mice were made to spin on a horizontal steel rod at 16 revolutions per minute (rpm) for five minutes. Mice who were able to maintain their position at the top for three minutes in three isolated chances were chosen for the Investigations.¹⁰⁻¹² There were six groups of animals total, with five mice in each group. The categories are mentioned below:

Group I: 2.5% tween 80 (Control group of animals received vehicle)

Group II: 2 mg/kg i.p (diazepam)

Group III: 200 mg/kg p.o. (Chloroform leaves extract of *P. bicalyculata* (R.) Nees)

Group IV: 400 mg/kg p.o. (Chloroform extract of leaves of *P. bicalyculata* (R.) Nees)

Group V: 200 mg/kg p.o. (Ethanol extract of leaves of *P. bicalyculata* (R.) Nees)

Group VI: 400 mg/kg p.o. (Ethanol extract of leaves of *P. bicalyculata* (R.) Nees)

A positive test resulted from the mouse's inability to stay on the rod for at least three minutes, and its fall in time was tracked every thirty to one hundred minutes.

Exploratory behavior pattern (Hole board test)

A scientific research method for measuring animal anxiety, stress, and emotionality is the hole-board test (HBT). It is well known in the area of behavioral pharmacology that this test exists. The hole board contraption is made out of a wooden box that is placed on the ground and has 16 evenly spaced holes, each measuring 3 cm.¹³ The device was raised to a height of 25 cm. The groupings fall under the categories mentioned in Motor coordination studies above.

Over the course of five minutes, the quantity of head pokes (head dipping) was counted. When both eyeballs vanished into the hole, the head dip was captured on camera.

Maximal electroshock induced convulsion

Grand mal epilepsy was effectively treated with electroconvulsive treatment. After 60 minutes of oral administration of plant extract, vehicle, or diazepam, the mice were subjected to a current of 150 mA for 0.2 seconds using an electroconvulsimeter equipped with ocular electrodes in order to induce tonic convulsions.¹⁴ Six groups of five mice each were created out of the animals. These are the groups:

Group I: 2.5 % tween 80 (Control group of animals received vehicle)

Group II: 2 mg/kg i.p (diazepam)

Group III: 200 mg/kg p.o. (Chloroform leaves extract of *P. bicalyculata* (R.) Nees)

Group IV: 400 mg/kg p.o. (Chloroform extract of leaves of *P. bicalyculata* (R.) Nees)

Group V: 200 mg/kg p.o. (Ethanol extract of leaves of *P. bicalyculata* (R.) Nees)

Group VI: 400 mg/kg p.o. (Ethanol extract of leaves of *P. bicalyculata* (R.) Nees)

The study monitored the presence and extent of extensor tonic postures. total absence of rear limb extension throughout the experiment was considered as complete protection against the medication's effect.

Light-dark test

Rats' natural aversion to highly lit environments and their impulsive exploratory activity in response to moderate stimuli serve as the foundation for the light/dark transition test. The apparatus utilized for the light/dark transition test was a cage with two identical chambers. One was lit, but the other was left in complete darkness.¹⁵ Six groups of five mice each were created out of the animals. These are the groups:

Group I: 2.5% tween 80 (Control group of animals received vehicle)

Group II: 2 mg/kg i.p (diazepam)

Group III: 200 mg/kg p.o. (Chloroform leaves extract of *P. bicalyculata* (R.) Nees)

Group IV: 400 mg/kg p.o. (Chloroform extract of leaves of *P. bicalyculata* (R.) Nees)

Group V: 200 mg/kg p.o. (Ethanolic extract of leaves of *P. bicalyculata* (R.) Nees)

Group VI: 400 mg/kg p.o. (Ethanolic extract of leaves of *P. bicalyculata* (R.) Nees)

After placing each animal separately in the middle of the light-dark room, recordings were taken for five minutes. The following parameters were noted: 1. the regularity of the tunnel crossings, or changes in illumination between compartments. 2. Total amount of time in the illuminated area.

RESULT AND DISCUSSION

An honest endeavor was made to explore the possible therapeutic advantages of stem bark extract derived from *P. bicalyculata* (R.) Nees. The study's positive results on the plant's conventional use provided scientific validation for the customs' claims. The study's results, outlined in this summary, provide insights into the plant's medicinal uses.

Extraction from Source Plant

The aqueous extract, petroleum ether, chloroform, and ethanol yielded 1.6, 2.68, 6.86, and 5.54%, respectively. The extraction of bark material using ethanol and water yielded the highest quantity of extracts, followed by extracts derived from petroleum ether and chloroform (Table 1).

Phytochemical Analysis

Analysis conducted using qualitative and quantitative methods. The plant extracts were examined to determine the likely existence of phytochemicals, which are responsible for the therapeutic characteristics of plant. *P. bicalyculata* (R.) It was determined whether contained any secondary metabolites, such as alkaloids, flavonoids, triterpenoids, sterols, tannins, and others (Table 2).¹⁶ The data is presented in tabular form.

The ethanol and aqueous extract was found to contain a diverse array of compounds shown in table. Quantitative analysis of phenolic content in the samples was conducted using a standard gallic acid curve, while the flavonoid content was determined using quercetin as the standard. The total phenolic content in the *P. bicalyculata* (R.) Nees extract was quantified and reported as the equivalent amount of gallic acid (EGA), yielding a value of 152.12 ± 0.079 per unit of dry extract. Similarly, the investigation of flavonoid substance, expressed

Table 1: Yield of *P. bicalyculata* stem bark extracts (% w/w)

Extract	Quantity used		Yield (%)
	Bark material (g)	Solvent (ml)	
Petroleum ether	100	500	1.6%
Chloroform	100	500	2.86%
Ethanol	100	500	6.86%
Aqueous	100	250	5.54%

Table 2: Qualitative phytochemical analysis of *P. bicalyculata* (R.) Nees extract

Qualitative test	<i>P. bicalyculata</i> (R.) Nees extract (PBA)
Terpenoids	+
Tannins	+
Saponins	+
Glycosides	+
Sterols	-
Alkaloids	+
Flavonoids	+
Carbohydrates	+
Proteins	-
Fats and oils	-
Organic acids	+
Coumarins	+
Quinine	+

as quercetin correspondent, revealed a concentration of $65.82 \pm 0.166 \mu\text{g mg}^{-1}$ in the dry extract of *P. bicalyculata* (R.) Nees.¹⁷

Neuropharmacological Activity¹⁸⁻²⁰*Enhancing the sleep duration produced by diazepam*

Current research demonstrates that chloroform and ethanolic extracts have ability to extend duration of sleep caused by diazepam, while also reducing the time it takes for diazepam to take effect. This effect is likely due to the extracts' impact on the brain systems that regulate sleep. The extracts of *P. bicalyculata* (R.) Nees exhibited a noteworthy CNS depressive action, with a considerable level of statistical significance ($p < 0.001$)(Table 3).

Spontaneous motor activity (SMA)

The locomotor activity exhibited a drop in count numbers that was depending on the dosage, as recorded on the Actophotometer. Therefore, the decrease in voluntary physical movement suggests that the extracts of *P. bicalyculata* (R.) Nees possess a noteworthy ($p < 0.001$) central nervous system depressive characteristic (Table 4).

Motor coordination

The values exhibit a substantial disparity with a significance level of $p < 0.01$. The use of the revolving bar is advantageous for detecting the muscle relaxation action of the test substance. Typical animals may be housed for an extended duration on

Table 3: Enhancing the sleep duration produced by diazepam

Group	OOA measured in minutes	DOA measured in minutes
Group I	7.65+1.17	54.67+5.71
Group II	3.14+0.80**	103.83+4.90**
Group III	6.42+0.83	52.42+2.14
Group IV	5.37+0.17	71.60+3.33
Group V	5.24+0.17*	74.66+5.16*
Group V: Ethanolic extract (400 mg/kg) + diazepam	4.53+0.38**	92.00+5.65**

OOA: Onset of action and DOA: Duration of action

the spinning rod. The motor activity exhibited substantial ($p < 0.001$) alterations when comparing the ethanolic extract at both dosage levels to the chloroform extract. The loss in motor coordination leads to the manifestation of the neuromuscular blocking characteristic (Table 5).

Hole board test (Exploratory behavior pattern)

Since there were 5 participants in the study, the findings are presented as the mean plus the standard error of the mean (SEM). The data was evaluated by comparing the observations to a standard group and then using a one-way ANOVA followed by Dunnett's test. At a significance level of $p < 0.001$, the values

Table 4: Spontaneous motor activity (SMA)

Group	0	30	60	90	120
Control	321.55+7.53	320.33+4.92	312.06+7.02	317.98+8.42	311.81+13.18
Vehicle and diazepam (2 mg/kg)	318.85+8.24	120.76+11.90**	60.33+7.01**	42.89+7.85**	65.68+14.74*
Chloroform extract (200 mg/kg)	325.78+4.24	226.23+4.72	148.41+12.8*	134.28+7.09	165.54+4.98
Chloroform extract (400 mg/kg)	329.25+4.42	187.06+7.04*	141.37+13.19	126.07+12.19	148.35+8.36
Ethanolic extract (200 mg/kg)	327.52+5.01	179.92+9.14*	128.52+26.44**	90.88+6.89	122.80+7.95
Ethanolic extract (400 mg/kg)	321.708+10.11	151.62+11.8**	113.57+7.01**	57.01+8.18**	110.26+10.81

Table 5: Motor coordination using rotarod apparatus

		Time spent on rods (5min)				
Group	Treatment	0	30	60	90	120
I	Control	216.46 ± 7.71	219.76 ± 5.60	214.24 ± 3.23	221.09 ± 4.11	223.54 ± 10.4
II	Vehicle and diazepam (2 mg/kg)	214.28 ± 7.02	50.28 ± 10.21**	26.49 ± 4.42**	74.36 ± 6.88**	107.73 ± 8.10**
III	Chloroform extract (200 mg/kg)	219.03 ± 4.17	133.94 ± 8.12	93.01 ± 11.52	127.51 ± 7.12	164.26 ± 7.83
IV	Chloroform extract (400 mg/kg)	221.63 ± 4.12	122.40 ± 14.15*	75.31 ± 11.31*	115.41 ± 8.32	152.95 ± 6.45
V	Ethanolic extract (200 mg/kg)	222.38 ± 8.31	86.77 ± 20.33	65.43 ± 9.41**	104.19 ± 8.12	125.74 ± 1.06
VI	Ethanolic extract (400 mg/kg)	214.03 ± 5.15	78.89 ± 50.11	46.04 ± 8.14**	89.53 ± 5.28**	116.31 ± 2.34

Table 6: Results of Hole board test

		Head dipping count within five min	
Group	T/T	Earlier of treatment	Later of treatment
I	Control	18.27 ± 2.38	18.27 ± 0.90
II	Vehicle + diazepam (2 mg/kg) i.p	18.27 ± 1.50	24.37 ± 1.53**
III	Chloroform extract of <i>P. bicalyculata</i> (R.) Nees p. o	16.24 ± 1.70	23.35 ± 1.16
IV	Chloroform extract of <i>P. bicalyculata</i> (R.) Nees (400 mg/kg) p. o	18.27 ± 1.60	25.38 ± 2.27*
V	Ethanolic extract of <i>P. bicalyculata</i> (R.) Nees (200 mg/kg) p. o	17.24 ± 1.75	29.44 ± 1.16**
VI	Ethanolic extract of <i>P. bicalyculata</i> (R.) Nees (400 mg/kg) p. o	19.32 ± 1.95	33.82 ± 2.07**

Table 7: Maximal electroshock induced seizures

Group	T/T	Period of tonic rear limb extension in seconds	Frequency of seizures
I	Control	28.75 ± 4.96	8/8
II	Vehicle + diazepam (2 mg/kg) i.p	5.08 ± 0.72**	2/8
III	Chloroform extract of <i>P. bicalyculata</i> (R.) Nees (200 mg/kg) p. o	27.41 ± 1.26	7/8
IV	Chloroform extract of <i>P. bicalyculata</i> (R.) Nees (400 mg/kg) p. o	25.89 ± 1.29*	6/8
V	Ethanolic extract of <i>P. bicalyculata</i> (R.) Nees (200 mg/kg) p. o	18.27 ± 1.60*	5/8
VI	Ethanolic extract of <i>P. bicalyculata</i> (R.) Nees (400 mg/kg) p. o	13.68 ± 0.96**	3/8

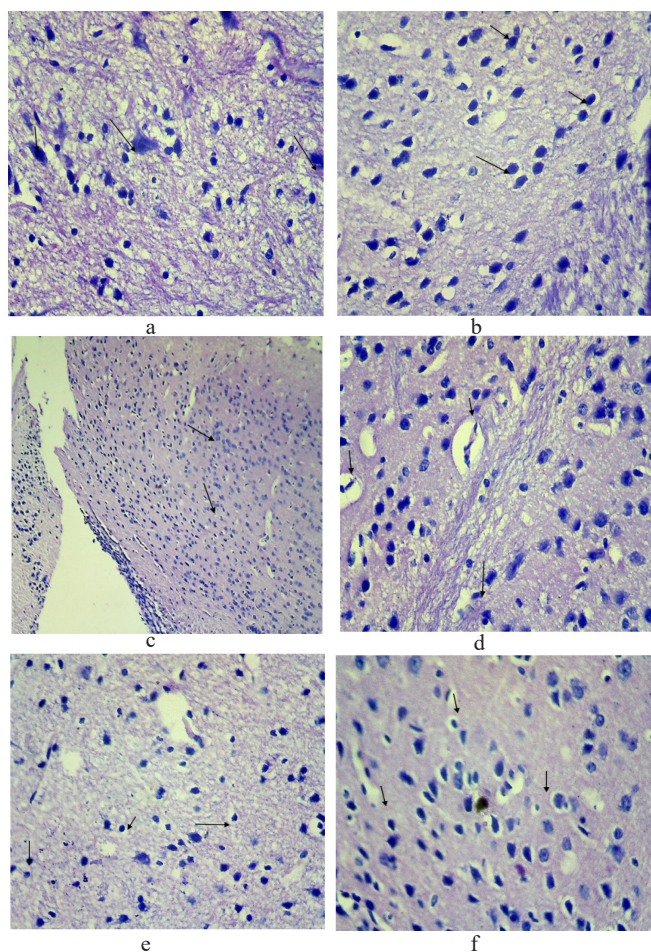


Figure 2: a) The brain tissue from the control group mouse exhibits typical histological features with no apparent neuronal damage; b) The brain tissue from the standard group mouse displays normal neuronal morphology; c) The brain tissue from the group treated with chloroform extract of *P. bicalyculata* (R.) Nees (CEDA) at a dose of 200 mg/kg exhibits histological features consistent with normal neuronal architecture; d) The brain tissue from the group treated with Chloroform extract of *P. bicalyculata* (R.) Nees (CEDA) at a dose of 400 mg/kg reveals histological characteristics indicative of normal neuronal morphology; e) The brain tissue from the group treated with ethanolic extract of *P. bicalyculata* (R.) Nees (EEDA) at a dose of 200 mg/kg demonstrates typical neuronal architecture; f) The brain tissue from the group treated with ethanolic extract of *P. bicalyculata* (R.) Nees (EEDA) at a dose of 200 mg/kg demonstrates typical neuronal architecture activity

show a major difference, whereas at a significance level of $p < 0.01$, the values show a significant difference. Anxiety may be effectively simulated in animals using the hole board test. A robust relationship between the animal's emotional state and the effects of *P. bicalyculata* (R.) Nees extracts was shown in the head dipping behavior test. Head dipping behaviors were significantly ($p < 0.001$) more common in the extract group as compared to the control group. Both of the *P. bicalyculata* (R.) Nees extracts were found to have anxiolytic effects in this investigation. When given at different doses, the chloroform and ethanolic extracts successfully reduced the convulsions caused by maximum electroshock. This may also indicate that extracts exhibit a very substantial ($p < 0.001$) anticonvulsant effect (Table 6).

Maximal electroshock induced seizures

Analysis are expressed as the mean \pm standard error of the mean (SEM) based on five data points obtained from six observations. One-way ANOVA was used to compare these measurements to a standard group. Subsequently, Dunnett's test identified statistically significant differences between individual groups and the standard. Significance levels are denoted by asterisks for $p < 0.01$ and double asterisks for $p < 0.001$ (Table 7).

Light-dark test

Anxiety often arises from an internal struggle between the natural urge to explore and the initial hesitation towards the unknown. In the light/dark test, assessing anxiety involves measuring the frequency of transitions into the light chamber and the total time spent there. Increased values in these measures are generally considered indicative of anxiolytic-like properties, signifying reduced anxiety. Notably, the extract of *P. bicalyculata* (R.) Nees demonstrated a remarkable anxiolytic effect, evidenced by a statistically significant ($p < 0.001$) increase in the time spent within the illuminated chamber, suggesting its potential to alleviate anxiety (Table 8).

We presented the means and standard error of variability (SEM) for five data points that were derived from six observations. To compare these values against a standard group, a one-way ANOVA test was conducted. Following this, Dunnett's test identified statistically significant differences between individual groups and standard. Double asterisks (**) denote the significance levels for $p < 0.001$, while single asterisks (*) for $p < 0.01$ are used.

Table 8: Light-dark test

Group	Treatment	Time spend in light area measured in seconds	Quantity of shift between light and dark area
I	Control	98.7 \pm 12.00	15.23 \pm 2.773
II	Vehicle and diazepam (2 mg/kg) i.p	188.9 \pm 9.31**	15.62 \pm 2.33**
III	Chloroform extract of <i>P. bicalyculata</i> (R.) Nees (200 mg/kg) p. o	131.7 \pm 7.97	25.38 \pm 1.61
IV	Chloroform extract of <i>P. bicalyculata</i> (R.) Nees (400 mg/kg) p. o	143.7 \pm 8.57	22.33 \pm 1.61
V	Ethanolic extract of <i>P. bicalyculata</i> (R.) Nees (200 mg/kg) p. o	159.3 \pm 7.07*	18.27 \pm 0.84**
VI	Ethanolic extract of <i>P. bicalyculata</i> (R.) Nees (400 mg/kg) p. o	165.7 \pm 8.33**	17.85 \pm 2.10**

Histopathological Report of Neuropharmacological Study

The mouse brain's histological analysis turned up no signs of glial proliferation or neuronal impairment. Siddha, Ayurveda, Unani, and Allopathy are just a few of the indigenous medical systems that make use of herbs and other plant-based remedies. The continued practice of the behaviors can be attributed to their significant impact on human flourishing, cultural beliefs, and biological advantages. The need to conserve the information ingrained in traditional healing techniques has sparked a growing interest in herbal medications. Use of herbal remedies as part of conventional medicinal methods has seen a sharp increase in popularity. These goods are also seen as essential to leading a healthy lifestyle and reducing dependency on traditional medical care. Each group's brain neurons underwent a histological evaluation, which is shown in the images (Figure 2a to 2f). The explanation is given in the paragraph that follows.

CONCLUSION

In southern India, *P. bicalyculata* (R.) Nees is commonly found as an ornamental plant, although its medicinal properties, including its purported efficacy in treating rheumatism, bronchitis, asthma, hepatic disorders, diabetes, and gonorrhea, lack substantial evidence. Nonetheless, it is utilized as an immunomodulator and as an antidote for snake venom. *P. bicalyculata* (R.) Nees extracts increased benzodiazepine sedation and decreased spontaneous motor activity, suggesting a central depressive impact, according to the study's conclusion. Furthermore, as evaluated by motor synchronization tests, the extracts had neuromuscular blocking characteristics and decreased motor coordination in mice. Furthermore, the extracts shortened the maximal electroshock seizures (MES) extension phase. Behavioral alterations indicative of anxiety were observed in mice subjected to the hole board and light/dark tests. Histological studies also supported alterations in brain activity. These findings collectively underscore the anticonvulsant, anxiolytic, and central nervous system (CNS) depressive properties of *P. bicalyculata* (R.) Nees' chloroform and ethanolic extracts. Future investigations will delve into the plant's potential interactions with neurotransmitters and elucidate its neurobiological mechanisms of action to identify the active ingredient responsible for these effects.

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