

Extraction, Isolation and Neuropharmacological Potential of *Phyllanthus acidus* L.

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Received: 18th December, 2023; Revised: 21st January, 2024; Accepted: 06th March, 2024; Available Online: 25th March, 2024

ABSTRACT

Because of their broad availability, low cost, low danger, and few side effects, medicinal herbs are vital in traditional medication all over the world. Traditional therapeutic herb *Phyllanthus acidus* L. is well-known for its calming possessions on the nervous system. Its roots are known for their activity against bacteria, inflammation, immunity stimulation, and erogenous properties, which contribute to potential health benefits. This study investigates the neuropharmacological effects of *P. acidus* L. in enhancing memory, targeting cognitive disorders and learning deficits. Analysis of the ethanolic extract's phytochemistry of *P. acidus* L. revealed a rich composition of phytoconstituents. UV analysis and thin layer chromatography (TLC) bioassay demonstrated significant acetylcholinesterase (AChE) inhibition by the extract. TLC and high-performance thin-layer chromatography (HPTLC) analyses of the extract indicated distinct and well-separated spots, suggesting the presence of active compounds. Additionally, the extract was assessed for its total phenolic, flavonoid, and ascorbic acid content, revealing antioxidant properties through *ex-vivo* lipid peroxidation assays. The study concludes that the ethanolic extract of *P. acidus* L. exhibits memory-enhancing, antidepressant, and neuroprotective effects attributed to its AChE inhibition, modulation of neurotransmitters like dopamine and serotonin, and antioxidant activity. This suggests its potential utility in Alzheimer's dementia treatment, though further research is necessary to elucidate pharmacology with mechanisms of action and applications.

Keywords: Extraction, Isolation, Neuropharmacological potential, Medicinal plants, *Phyllanthus acidus* L.

International Journal of Drug Delivery Technology (2024); DOI: 10.25258/ijddt.14.1.43

How to cite this article: Khare N, Tare H, Jain SK. Extraction, Isolation and Neuropharmacological Potential of *Phyllanthus acidus* L. International Journal of Drug Delivery Technology. 2024;14(1):299-305.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

A group of neurological disorders known as conditions influencing the central nervous system (CNS) impact the structure and/or function of the spinal cord and/or brain. Parkinson's disease, insomnia, anxiety, melancholy, epilepsy, dementia, pain, schizophrenia, psychosis, and stroke are among the conditions included. They account for significant worldwide loss of life and disability. CNS disorders are placing an increasing strain on healthcare systems as a result of population growth and aging. There will likely be a 12.3 to 15% increase in the prevalence of illnesses affecting CNS by 2020. The hunt for new bioactive molecules is critical because most medications used to treat CNS problems have a number of side effects and don't work as advertised. Scientists have historically depended on natural resources in order to formulate groundbreaking pharmaceuticals that address the current health demands of the human population. A multitude of botanical species have exhibited neuropharmacological properties, and current research endeavors are focused on exploring minimally invasive methods to treat disorders affecting the central nervous system.^{1,2}

A little, flat tree that can grow to be up to 10 meters tall is the *Phyllanthus acidus*. Prominent lenticels and gritty, gray bark identify this plant as having phyllanthoid branching. The plant exhibits transient cataphylls that are dark brown in color and have triangular-ovate stipules. Climbing deciduous branchlets of this plant contain 25 to 40 leaflets. The pinnate leaves measure 20 to 40 cm in length. The leaflets are undivided, elongated to elongated-lanceolate in form, stalk-attached, and have a pointed apex and a blunt to rounded base. The length and breadth of the object vary between 4 and 5 cm, and 2 and 9 cm, respectively. The range of measurement is 4.5 to 2.5 cm. The stipules are 2.5 to 4 mm in length and are triangular-acuminate.³

In Nigeria, paralysis, convulsions, fevers, epilepsy, and other neurological ailments are treated with concoctions made from the leaves. The ethyl acetate fraction (EF) contained an identical composition to the methanol extract (ME), excluding terpenoids and steroids. Glycosides, saponins, flavonoids, alkaloid substances, terpenoids, tannins, and steroids were discovered in the first phytochemical studies of the ME.⁴ Previous research has demonstrated that various components

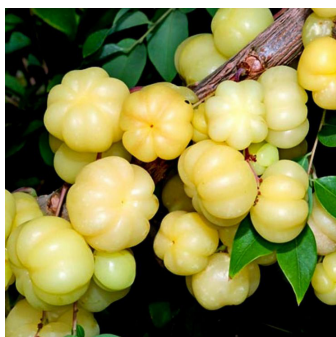


Figure 1: Stem bark of *P. acidus* along with fruits

of plant, specifically its leaves, exhibit properties that inhibit the growth of bacteria, yeast, and lipids, are antimicrobial, immunostimulatory, antifungal, and antidiarrheal. According to reports, aqueous extract obtained from aerial portions of plants has demonstrated potential benefits in the treatment of metabolic, reproductive, and pain management challenges. Nevertheless, no empirical evidence supports its influence on neuropharmacological effects. In the past, many chemicals were isolated and identified from the plant's leaves. These substances included methyl gallate, 2-ethyloctylphthalate, 3-friedelanone, ellagitannin, bis 2-ethylcosylphthalate, geraniin, gallic acid, and kaempferol glycosides. This investigation endeavor aimed to impartially assess the neuropharmacological effects of ethyl acetate fraction (EF) and methanol leaf extract (ME) of *P. acidus* in mice. Based on ethnobotanical data and multiple models, investigation indicates that PA may be utilized to treat convulsions and other diseases of the central nervous system (CNS).^{5,6}

MATERIAL AND METHODS

Material and Extraction of Plant

In order to maintain the stem bark's integrity, it was carefully cleaned and then dried in the shade (Figure 1). Once dried, it was mechanically pulverized and passed through a sieve with a mesh size of 10/44 to ensure uniform particle size.

Petroleum ether was used to defat the extract using a Soxhlet device. In order to obtain the ethanol extract, the defatted material (600 g) was subjected to hot extraction in two stages: the first, with 1.5 L of chloroform at 45°C for approximately 15 cycles, and the second, with 2 L of ethanol at 50°C for around 15 to 17 cycles. The aqueous extract was obtained by heating 100 g of plant material in 200 mL of water to 65°C for 15 minutes and then filtering the mixture. The samples were air-dried. Desiccators were used to prevent the oxidation of the ethanolic extract and water extract of *P. acidus* (PAE) until the next round of research.

Phytochemical Analysis

A qualitative examination of *P. acidus* (PAE) crude extract phytochemicals showed that the plant stores many phytochemicals. These extracts included polar phytochemicals. The current research shows that *P. acidus* (PAE) stem bark contains active phytochemicals that benefit the plant.^{7,8}

Test for alkaloids

After reconstituting the extracts, a diluted hydrochloric acid solution was added, mixed thoroughly, and filtered. The extract is tested by Mayer's, Wagner's, Hanger's, and Dragendorff's tests.

Test for flavonoids

Flavonoid is tested by ferric chloride, zinc-hydrochloric acid reduction, Shinoda, alkaline reagent and lead acetate solution test.

Test for triterpenoids

Triterpenoids are tested by the Salkowski, Liebermann-Burchard test.

Test for sterols

Salkowski, Liebermann-Burchard and sulphur test test sterols.

Test for quinones

Quinones are tested by potassium iodide test.

Test for other phytoconstituents

The following solutions are used for tannin testing: About 5% ferric chloride and 10% lead acetate; foam for saponin testing; 10% sodium hydroxide for coumarins; Molisch for carbohydrates; and a small amount of a 5% calcium chloride solution for organic acids.⁹⁻¹³

Neuropharmacological Activity

General behaviour studies

Behavioral evaluation was the method used in this research, which included dividing the albino Wistar rats in 5 groups of six rats. The first three groups received oral doses of 50, 100, and 200 mg/kg of *P. acidus* L. ethanolic extract, respectively. A positive drug control with 5 mg/kg of diazepam and a vehicle control with 2% v/v tween 80 were given to the other two groups. With an emphasis on predetermined criteria, behavioral changes were tracked at 30-minute intervals for the first hour and hourly intervals for the subsequent four hours.¹⁴⁻¹⁷

• Awareness, alertness and spontaneous activity

The animal's responsiveness to its environment and its capacity to go around obstacles and stumbling-free were used to visually evaluate its level of awareness and alertness. Many animals display a mild degree of interest.

• Righting reflex

The test chemicals were administered to the rats on the designated test day. At time intervals of 15, 30, and 60 minutes, every rat was carefully positioned in a supine position on a corrugated surface composed of white iron and maintained at a temperature of 30°C. If the animal maintained a supine position for 30 seconds, it was deemed to have lost its righting reflex.

• Pinna reflex

A hair or other thin object is used to apply light pressure to the middle of the pinna in order to test the response.

- *Grip strength*

The grasp strength test is utilized in rodents to evaluate neuromuscular function or muscular strength. For this measurement, we let the animal hold a pencil laid flat on the table and timed how long it took for the pencil to drop to the floor.

- *Touch response*

Using a pencil or forceps to press on different parts of the rat's body (like the pelvis, abdomen, and side of the neck) to record the contact reaction.

- *Pain response*

A lesser blood vessel tourniquet was put on the bottom of the tail and the pain response was measured by writing down the reaction.

- *Sound response*

Since albino wistar rats typically remain silent, vocalization could be an indication of an aversive stimulus.

Spontaneous motor activity (SMA)

The Digital Actophotometer was used to determine how much the animals moved independently. 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.¹⁸ The mice were split up in six clusters, every consisting of 5 mice. Following are groupings:

- 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 and fruits extract of *P. acidus* L.)
- Group IV: 400 mg/kg p.o (Chloroform extract of leaves and fruits of *P. acidus* L.)
- Group V: 200 mg/kg p.o. (Ethanol extract of leaves and fruits of *P. acidus* L.)
- Group VI: 400 mg/kg p.o. (Ethanol extract of leaves and fruits of *P. acidus* L.)

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 that were able to maintain their position at the top for three minutes were chosen for the investigation. Nearby existed six clusters of animals total, with five mice in each group. The categories for the groups are as follows:

- 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 and fruits extract of *P. acidus* L.)
- Group IV: 400 mg/kg. p.o (Chloroform extract of leaves

and fruits of *P. acidus* L).

- Group V: 200 mg/kg. p.o. (Ethanol extract of leaves and fruits of *P. acidus* L.)
- Group VI: 400 mg/kg.p.o. (Ethanol extract of leaves and fruits of *P. acidus* L.)

Exploratory behavior pattern (Hole board test)

A scientific research method for measuring animal anxiety, stress, and emotionality is the hole-board test (HBT). This test is well known in the area of behavioral pharmacology. The hole board device comprises a woody container on the ground with 16 uniformly distributed holes, each with a diameter of 3 cm. The apparatus was elevated to a 25 cm height.¹⁹ There were six groups of animals total, with 5 mice in individual group. Categories 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 and fruits extract of *P. acidus* L.)
- Group IV: 400 mg/kg. p.o (Chloroform extract of leaves and fruits of *P. acidus* L.)
- Group V: 200 mg/kg. p.o. (Ethanol extract of leaves and fruits of *P. acidus* L.)
- Group VI: 400 mg/kg.p.o. (Ethanol extract of leaves and fruits of *P. acidus* L.)

Maximal electroshock induced convulsion

The electroconvulsive therapy was effective in treating grand mal epilepsy. In order to produce tonic convulsions in mice, an electro-convulsimeter with ocular electrodes was used after 60 minutes after intramuscular (IM) administration of an herbal extract, vehicle, or standard. Each of these categories:

- 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 and fruits extract of *P. acidus* L.)
- Group IV: 400 mg/kg. p.o (Chloroform extract of leaves and fruits of *P. acidus* L.)
- Group V: 200 mg/kg. p.o. (Ethanol extract of leaves and fruits of *P. acidus* L.)
- Group VI: 400 mg/kg p.o. (Ethanol extract of leaves and fruits of *P. acidus* L.)

Light-dark test

Rats' innate dislike of highly illuminated settings and their propensity for exploratory activity in response to moderate stimuli. The test equipment contained of a box with 2 similar chambers separated by a barrier. There was light in one compartment and darkness in the other.²⁰ Here 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 and fruits extract of *P. acidus* L.)
- Group IV: 400 mg/kg. p.o (Chloroform extract of leaves

and fruits of *P. acidus* L).

- Group V: 200 mg/kg. p.o. (Ethanol extract of leaves and fruits of *P. acidus* L)
- Group VI: 400 mg/kg p.o. (Ethanol extract of leaves and fruits of *P. acidus* L.)

RESULT AND DISCUSSION

An earnest effort was exerted to investigate the potential medicinal value of stem bark extract of *P. acidus* L. (PAE). The research yielded encouraging findings regarding the traditional applications of the plant, thereby substantiating the traditional assertions with scientific evidence. A tabulation of the study's findings provides insight into the pharmacological applications of the plant.

Extraction from Source Plant

The results obtained from the aqueous, chloroform, ether, and petroleum ether extracts were as follows: 0.6, 1.68, 7.16, and 6.64%, respectively (Table 1). The results show that the bark material was extracted with the highest quantity of extracts using ethanol and water, followed by chloroform and petroleum ether.

Phytochemical Analysis

Analysis, both qualitative and quantitative phytochemicals, the active ingredients in plants that have therapeutic effects, were searched for in the plant extracts (Table 2). Secondary metabolites were examined in *P. acidus* L. extract. What follows is a table detailing the findings.

HPLC-UV Analysis

Assessing the antioxidant potential of medicinal plants requires the study of phenolics and flavonoids, which is considered significant. The antioxidant compounds included in the extract of *P. acidus* L. were discovered using high-performance liquid chromatography (HPLC) in comparison to other standard phenolic and flavonoid molecules. By examining the retention durations of standard molecules and the UV spectra of these peaks under the same experimental settings, it is evident that there are significant amounts of antioxidant phenolic acids, as shown in Table 3.

P. acidus L. extract's UV spectral peaks at 350 nm indicated that luteolin, myricetin, quercetin, and rutin were present in the PAE. Chromatographic examination of spike extracts, in contrast to the usual phenolic and flavonoid components, may have uncovered the existence of gallic acid, ellagic acid, hydroxyl benzoic acid, coumaric acid, rutin, quercetin, myricetin, and luteolin. The existence of many and

Table 2: Qualitative phytochemical analysis of *P. acidus* L. extract

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

significant antioxidant components may be the cause of the protective and antioxidant properties of the *P. acidus* L. extract. At a wavelength of 220 nm, three unexplained peaks, gallic acid, ellagic acid, and hydroxybenzoic acid were detected in PAE, with retention periods of 1.96, 5.68, and 8.27 minutes, respectively. Gallic acid was the most common phenolic component in PAE, with a 121.3 mg g⁻¹ concentration.

Isolation of Bioactive Compound

Phytochemistry has enabled the separation and identification of several beneficial compounds. Starting with the identification of the alkaloid quinine, the list comprises an endless array of substances. A multitude of phytochemicals have been categorized, with over 150 of them being extensively studied. As part of the ongoing search for new medical treatments, it has become common practice to isolate chemical molecules and determine their structures using various spectroscopic methods. If considered appropriate, gas chromatography–mass spectrometry (GC-MS) may be used to describe the preparations. This offers a comprehensive understanding of the compound's structure and its qualities. After identifying an active extract, the first step is ascertaining the bioactive phytochemicals. This may include the thorough identification of the bioactive phytochemicals after purification or their partial identification up to the level of a known compound family. The goal is to isolate components from the bark of

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

Extract	Quantity used		Yield (%)
	Bark material (g)	Solvent (mL)	
Petroleum ether	100	500	0.6
Chloroform	100	500	1.68
Ethanol	100	500	7.16
Aqueous	100	250	6.64

Table 3: Quantitative HPLC analysis of PAE

Standard	Phenolic compounds	Standard	Flavonoids PAE (mg g ⁻¹)
	PAE (mg g ⁻¹)		
Vanillic acid	-	Luteolin	9.69
Coumaric acid	-	Quercetin	26.02
Hydroxyl benzoic acid	53.71	Kaempferol	-
Ellagic acid	15.57	Myricetin	7.868
Gallic acid	121.3	Rutin	52.10



Figure 2: Thin layer chromatography of extract of *P. acidus* L. in CHCl_3

Table 4: Rf Value of extracts of *P. acidus* L.

S. No	Extracts	Rf value
1	<i>P. acidus</i> L.	0.88

P. acidus L. The extract was confirmed via the use of thin-layer chromatography (TLC) (Figure 2, Table 4) after it was produced using polarity gradient column chromatography. Consequently, the process produced a chemical fraction and two hydrocarbon fractions.²¹

Retention factor = Distance moved by the *P. acidus* L. components/Distance moved by the selected solvent
 = 5.4/6.1
 = 0.88

Neuropharmacological Activity

Spontaneous motor activity

Results are depicted as mean \pm SEM, with a sample size of $n = 5$, derived from six observations (Table 5). One-way ANOVA and Dunnett's test were applied, and these were contrasted with the control group. Values were deemed significant when $p < 0.01$.

Motor coordination

Results of six observations are shown as mean + SEM, ($n = 5$). One-way ANOVA and Dunnett's test were applied, and these were contrasted with the control group. Values were deemed significant when $p < 0.01$ (Table 6).

Exploratory behavior pattern (Hole board test)

Five observations were compared to a standard group using one-way ANOVA. For each observation, the average value (mean) and variability (SEM) were reported (Table 7). Using Dunnett's test, we could identify significant differences between the standard group and individual observations; asterisks denote statistically significant differences at 1% and stars at 5%.

Maximal electroshock induced convulsion

The results of the analysis are stated as mean \pm standard error of the mean (SEM) based on five data points obtained from six observations (Table 8). 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. For $p < 0.01$, asterisks indicate significance levels; for $p < 0.001$, two asterisks are used.

Table 5: Spontaneous motor activity

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

Table 6: Motor coordination

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

Table 7: Exploratory behavior pattern (Hole board test)

Group	T/t	Head dipping count within five min	
		Earlier treatment	Later 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. acidus</i> L (200 mg/kg) p. o	16.24 ± 1.70	23.35 ± 1.16
IV	Chloroform extract of <i>P. acidus</i> L (400 mg/kg) p. o	18.27 ± 1.60	25.38 ± 2.27*
V	Ethanol extract of <i>P. acidus</i> L (200 mg/kg) p. o	17.24 ± 1.75	29.44 ± 1.16**
VI	Ethanol extract of <i>P. acidus</i> L (400 mg/kg) p. o	19.32 ± 1.95	33.82 ± 2.07**

Table 8: Maximal electroshock induced convulsion

Group	Treatment	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>Peristrophe bicalyculata</i> (R.) Nees and <i>P. acidus</i> L (200 mg/kg) p. o	27.41 ± 1.26	7/8
IV	Chloroform extract of <i>P. bicalyculata</i> (R.) Nees and <i>P. acidus</i> L (400 mg/kg) p. o	25.89 ± 1.29*	6/8
V	Ethanol extract of <i>P. bicalyculata</i> (R.) Nees and <i>P. acidus</i> L (200 mg/kg) p. o	18.27 ± 1.60*	5/8
VI	Ethanol extract of <i>P. bicalyculata</i> (R.) Nees and <i>P. acidus</i> L (400 mg/kg) p. o	13.68 ± 0.96**	3/8

Table 9: Light-dark test

Group	Treatment	Period spent in light zone (sec)	Total of switches between light and dark
I	Control	98.7 ± 12.00	15.23 ± 2.773
II	Vehicle and diazepam (2 mg/kg) i.p	188.9 ± 9.31**	15.62 ± 2.33**
III	Chloroform extract of <i>P. acidus</i> L (200 mg/kg) p. o	131.7 ± 7.97	25.38 ± 1.61
IV	Chloroform extract of <i>P. acidus</i> L (400 mg/kg) p. o	143.7 ± 8.57	22.33 ± 1.61
V	Ethanol extract of <i>P. acidus</i> L (200 mg/kg) p. o	159.3 ± 7.07*	18.27 ± 0.84**
VI	Ethanol extract of <i>P. acidus</i> L (400 mg/kg) p. o	165.7 ± 8.33**	17.85 ± 2.10**

Light-dark test

Five data points, obtained from six observations, were analyzed, and their average values (means) and variability (standard error of the mean, SEM) were reported (Table 9). A one-way ANOVA test was conducted to compare these values against a standard group. Following this, Dunnett's test identified statistically significant differences between individual groups and the standard. For $p < 0.001$, the significance levels are denoted by two asterisks and for $p < 0.01$ by one asterisk (*).

DISCUSSION

Through several neuropharmacological situations, this study investigated the effects of PA leaf extracts on mice's central nervous system. Current anticonvulsant medications have serious side effects that most patients find unacceptable. Furthermore, although these medications may mitigate seizures, they do not provide a remedy. This has stimulated interest in exploring new botanical species that might potentially be used in treating epilepsy. Dread is a prevalent

manifestation of anxiety and anxiety-related disorders, which are neurological ailments that often lead to feelings of sadness. Sedation is a common adverse effect of anxiolytic medicines often used for these diseases. The current investigation set out to investigate the effects of PA on mouse central nervous system, with a focus on motor coordination, anxiety levels, and convulsion events. The therapeutic index (LD_{50}/ED_{50}) is a valuable tool for assessing the acute toxicity of medicines and xenobiotics. Based on the current investigation, the extract has an oral LD_{50} of > 5000 mg/kg, indicating that it is very unlikely to produce acute intoxication and may be considered safe.²²

CONCLUSION

The results raise the possibility that *P. acidus* L.'s methanol leaf extract has calming, anxiety-reducing, and anticonvulsant effects. There are no sedative or cognitive impairment effects from the extract's ethyl acetate fraction, which has anticonvulsant and anxiolytic properties. Taken together, our results highlight the possibility of developing agonists

with anticonvulsant and anxiolytic capabilities, free from the sedative side effects commonly associated with diazepam and other non-selective GABA agonists. Possible candidates for the development of insomnia therapies that contain the sedative components found in ME might be studied. In our upcoming studies, we want to isolate the bioactive ingredient or ingredients and identify the exact process by which it exerts its effects.

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