

PHYTOCHEMICAL CHARACTERIZATION AND ASSESSMENT OF ANXIOLYTIC POTENTIAL OF ETHANOLIC EXTRACT OF *PERGULARIA DAEMIA*

Uttam Kumar Nayak*, Satkar Prasad

RKDF School of Pharmaceutical Sciences, Bhabha University, Bhopal (M.P.)

*Corresponding Author's E mail: nayaku350@gmail.com

Received 12 June 2026; Revised 15 June 2026; Accepted 18 June 2026, Available online 15 July 2026



Cite this article as: Nayak U.K. and Prasad S. Phytochemical Characterization and Assessment of Anxiolytic potential of Ethanolic extract of *Pergularia daemia*, Asian Journal of Pharmaceutical Education and Research. 2026; 15(3): 92-102.

<https://dx.doi.org/10.38164/AJPER/15.3.2026.92-102>

ABSTRACT

Pergularia daemia is a medicinal plant widely used in traditional systems of medicine for the treatment of various ailments owing to its rich phytochemical composition and diverse pharmacological properties. The present study was undertaken to investigate the phytochemical profile and anxiolytic activity of the hydroalcoholic extract of *Pergularia daemia*. The hydroalcoholic extraction yielded 10.5% (w/w) extract. Preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, phenolic compounds, carbohydrates, diterpenes, and sterols, while glycosides, saponins, proteins, and tannins were absent. Quantitative estimation revealed total phenolic, flavonoid, and alkaloid contents of 0.67, 0.81, and 0.25 mg/100 mg of extract, respectively. The anxiolytic activity of the extract was evaluated in experimental animals using the staircase test and elevated plus-maze (EPM) model. The extract was administered orally at doses of 200 and 300 mg/kg, while diazepam (4 mg/kg, i.p.) was used as the standard drug. In the staircase test, the extract produced a significant and dose-dependent reduction in climbing and rearing behavior compared with the control group, indicating decreased anxiety-related exploratory activity. In the elevated plus-maze test, treatment with the extract significantly increased the number of entries into the open arms and the time spent in the open arms while reducing the number of closed-arm entries. The 300 mg/kg dose produced more pronounced anxiolytic effects than the 200 mg/kg dose and showed activity comparable to diazepam. The observed pharmacological activity may be attributed to the presence of flavonoids, phenolic compounds, alkaloids, and other bioactive phytoconstituents, which are known to modulate central nervous system function through antioxidant and GABAergic mechanisms. The findings of the present investigation scientifically support the traditional use of *Pergularia daemia* in the management of anxiety and suggest that the plant possesses considerable potential for the development of safe and effective herbal anxiolytic formulations. Further studies are warranted to isolate the active constituents, elucidate their mechanisms of action, and evaluate their long-term safety and clinical efficacy.

Keywords: *Pergularia daemia*; Hydroalcoholic extract; Anxiety; Anxiolytic activity; Elevated plus-maze; Staircase test; Phytochemical screening; Flavonoids; Phenolic compounds; Alkaloids.

INTRODUCTION

Anxiety disorders are among the most common psychiatric illnesses worldwide, affecting millions of people and significantly impairing quality of life, social functioning, and productivity ¹. These disorders are characterized by excessive fear, apprehension, restlessness, and autonomic disturbances that persist beyond normal adaptive responses to stress. Although conventional anxiolytic drugs such as benzodiazepines, selective serotonin reuptake inhibitors (SSRIs), and serotonin–norepinephrine reuptake inhibitors (SNRIs) are effective in managing anxiety, their long-term use is often associated with adverse effects including sedation, cognitive impairment, tolerance, dependence, withdrawal symptoms, and other undesirable complications. Consequently, there is growing interest in identifying safer and more effective therapeutic agents from natural sources ².

Medicinal plants have served as valuable sources of bioactive compounds for the treatment of neurological disorders for centuries. Numerous phytochemicals, particularly flavonoids, alkaloids, phenolic compounds, terpenoids, and sterols, have demonstrated significant neuroprotective, antioxidant, anti-inflammatory, and anxiolytic properties in experimental studies ³.

These compounds are capable of modulating neurotransmitter systems, including γ -aminobutyric acid (GABA), serotonin, dopamine, and glutamate pathways, thereby influencing anxiety-related behavior. Herbal medicines are generally considered to have fewer adverse effects and better patient acceptance than many synthetic drugs, making them attractive alternatives for the management of anxiety disorders⁴. *Pergularia daemia* (Forssk.) Chiov., belonging to the family Apocynaceae (formerly classified under Asclepiadaceae), is a perennial twining medicinal herb distributed throughout tropical and subtropical regions of Asia and Africa ⁵.

The plant has been extensively used in traditional systems of medicine for the treatment of fever, inflammation, rheumatism, asthma, diabetes, gastrointestinal disorders, skin diseases, and various nervous system ailments⁶. Phytochemical investigations have revealed the presence of several biologically active constituents, including flavonoids, alkaloids, phenolic compounds, steroids, terpenoids, and other secondary metabolites that contribute to its broad spectrum of pharmacological activities. Previous pharmacological studies have demonstrated antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, antidiabetic, analgesic, and wound-healing activities of *Pergularia daemia*. However, scientific evidence regarding its anxiolytic potential remains limited ⁷.

Oxidative stress and neuroinflammation have been recognized as important contributors to the pathophysiology of anxiety disorders. Plant-derived antioxidants may reduce neuronal oxidative damage and improve neurotransmitter balance, thereby exerting beneficial effects on anxiety-related behavior. Flavonoids and phenolic compounds, in particular, have been reported to interact with the

benzodiazepine-binding site of GABA receptors, producing anxiolytic effects comparable to standard drugs while minimizing adverse reactions. Therefore, medicinal plants rich in these phytoconstituents represent promising candidates for the development of novel anxiolytic agents⁸.

In view of these considerations, the present study was designed to evaluate the phytochemical composition and anxiolytic activity of the hydroalcoholic extract of *Pergularia daemia*. Preliminary phytochemical screening and quantitative estimation of total phenolic, flavonoid, and alkaloid contents were performed to characterize the extract. The anxiolytic potential was assessed using the staircase test and the elevated plus-maze model, two well-established experimental paradigms for evaluating anxiety-related behavior in rodents. Diazepam was employed as the reference standard for comparison. The findings of this study are expected to provide scientific evidence supporting the traditional use of *Pergularia daemia* and contribute to the development of plant-based therapeutic agents for the management of anxiety disorders.

MATERIAL AND METHODS

Material

The whole plant of *Pergularia daemia* was collected, authenticated, shade dried, powdered, and used for preparation of the hydroalcoholic extract. Analytical grade ethanol and distilled water were employed as extraction solvents. Standard phytochemical reagents, including Wagner's reagent, Hager's reagent, Legal's reagent, lead acetate, alkaline reagent, ferric chloride, Fehling's solution, copper acetate, gelatin solution, and Salkowski reagent, were used for qualitative phytochemical screening. Gallic acid, quercetin, and atropine were used as reference standards for the estimation of total phenolic, flavonoid, and alkaloid contents, respectively. Diazepam injection (4 mg/kg) served as the standard anxiolytic drug for pharmacological evaluation. All chemicals and reagents used in the study were of analytical reagent (AR) grade and procured from reputed commercial suppliers.

Methods

Extraction by maceration process

Extraction is an essential step in phytochemical following procedure will be adopted for the preparation of extract from the shade dried material⁹. 50 gram of shade dried aerial parts of *Pergularia daemia* were coarsely powdered and subjected to extraction with petroleum ether in a maceration method. The extraction was continued till the defatting of the material had taken place. Defatted plant materials of *Pergularia daemia* were exhaustively extracted with hydroalcoholic solvent (ethanol: aqueous: 80:20v/v) by maceration method. The extract was evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extracts.

Determination of percentage yield

After extraction, yield of the plant extracts obtained were calculated in grams and then converted it into percentage. The percentage yield of extract was calculated by using following formula:

$$\text{Percentage Yield} = \frac{\text{Weight of Extract}}{\text{Weight of Powder drug taken}} \times 100$$

Phytochemical screening

Medicinal plants are resources of traditional medicines and many of the modern medicines are produced indirectly from plants. Phytochemical constituents are of two type primary bioactive constituents (chlorophyll, proteins, amino acids, sugar etc.) and secondary bioactive constituents include (alkaloids, phenols, flavonoids etc.). Phytochemical examinations were carried out for all the extracts as per the standard methods ¹⁰.

Quantitative estimation of bioactive compounds

Estimation of total phenol content

The total phenolic content of the extract was determined by the modified folin-ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of folin-ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/L) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer ¹¹.

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method ¹². 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% AlCl₃ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

Estimation of total alkaloids content

The determination of total alkaloid content is a crucial step in assessing the alkaloid richness and potential pharmacological activities of plant extracts ¹³. The plant extracts (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with

chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract.

***In vivo* anti-anxiety activity of *Pergularia daemia* extract**

Acute toxicity studies

Acute toxicity studies were carried out using acute toxic class method as per OECD guidelines 425. Acute toxicity for hydroalcoholic extract of *Pergularia daemia* was carried out using groups of three Swiss albino mice by administering a dose 2000 mg/kg, in 1% CMC p.o., while the control group received only the vehicle. The groups were observed mortality and behavioral changes during 48 h.

Dosing and grouping of Animals

Swiss albino mice were taken and divided into four groups, each group comprised of 6 animals. The two doses of hydroalcoholic extract of *Pergularia daemia* (200 and 300 mg/kg) were administered orally, the standard group was treated with diazepam (4 mg/kg) intraperitoneally and control group received Tween 80 (2% w/v) orally.

Staircase test

Staircase consists of five identical steps 2.5 cm high, 10 cm wide and 7.5 cm deep. The internal height of the walls is constant along whole length of the staircase. The animals were placed on the floor of the box with its back to the staircase. The number of steps climbed and the number of rears were counted over a 3 min period. A step was considered to be climbed only if the mouse had placed all four paws on the step. In order to simplify the observation, the numbers of steps descended were not taken into account. After each step the box was cleaned in order to eliminate any olfactory cues, which might modify the behavior of the next animal ¹⁴.

Elevated plus maze

The apparatus consist of two open arms (5 × 10 cm) and two closed arms (5 × 10 × 15 cm) radiating from a platform (5 × 5 cm) to form a plus-sign figure. The apparatus was situated 40 cm above the floor. The open arms edges were 0.5 cm in height to keep the mice from falling and the closed-arms edges were 15 cm in height. The animal was placed at the center of the maze, facing one of the closed arms ¹⁵. During 5 min test period the following measures are taken:

- The number of entries into open arms
- The number of entries into closed arms

- Time spent in the open arms

Arm entry was counted when the animal had placed all of its four paws on it. The procedure was conducted in a sound attenuated room, with observations made from an adjacent room.

Statistical analysis

Results were expressed as Mean $\pm$ SEM the differences between experimental groups were compared using one-way Analysis of Variance (ANOVA) followed by Dennett's test and were considered statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

The present study was undertaken to investigate the phytochemical constituents and anxiolytic potential of the hydroalcoholic extract of *Pergularia daemia*. The extraction process yielded 10.5% (w/w) extract, indicating efficient extraction of polar and moderately polar phytoconstituents using the hydroalcoholic solvent system. The extraction yield obtained was satisfactory and comparable with previous reports on hydroalcoholic extraction of medicinal plants, demonstrating that the selected extraction method was suitable for recovering bioactive compounds from *Pergularia daemia*.

Preliminary phytochemical screening revealed the presence of several important secondary metabolites, including alkaloids, flavonoids, phenolic compounds, carbohydrates, diterpenes, and sterols, whereas glycosides, saponins, proteins, and tannins were absent. These phytochemicals are widely recognized for their diverse pharmacological activities, particularly their antioxidant, anti-inflammatory, neuroprotective, and central nervous system modulatory effects. Flavonoids and phenolic compounds have attracted considerable attention because they are capable of crossing the blood-brain barrier and modulating neurotransmitter systems involved in anxiety regulation. Similarly, alkaloids have been reported to interact with various neurotransmitter receptors, contributing to anxiolytic and sedative activities.

Quantitative phytochemical estimation demonstrated that the hydroalcoholic extract contained 0.67 mg/100 mg of total phenolic content, 0.81 mg/100 mg of total flavonoid content, and 0.25 mg/100 mg of total alkaloid content. The comparatively higher flavonoid content suggests that flavonoids may be one of the principal bioactive constituents responsible for the observed pharmacological effects. Phenolic compounds contribute significantly through their antioxidant activity by scavenging reactive oxygen species, thereby protecting neuronal tissues from oxidative stress, which is known to be associated with anxiety disorders. The presence of alkaloids further supports the neuropharmacological potential of the extract through possible interactions with GABAergic and monoaminergic neurotransmission.

The anxiolytic activity of the hydroalcoholic extract was evaluated using the staircase test, a validated behavioral model used to assess exploratory behavior and anxiety in rodents. Animals treated with the extract exhibited a significant reduction in both climbing and rearing activities compared with the control group. The effect was dose dependent, with the 300 mg/kg dose producing a greater reduction than the 200 mg/kg dose. Diazepam, used as the standard anxiolytic agent, produced the greatest reduction in exploratory activity. The decrease in climbing and rearing responses indicates suppression of anxiety-related behavior and suggests that the extract possesses central nervous system depressant and anxiolytic properties without producing abnormal behavioral responses.

The elevated plus-maze (EPM) test further confirmed the anxiolytic activity of the extract. Anxiety in rodents is characterized by avoidance of open and elevated spaces; therefore, increased exploration of the open arms is considered a reliable indicator of anxiolytic activity. In the present study, administration of the hydroalcoholic extract significantly decreased the number of entries into the closed arms while increasing both the number of entries into the open arms and the time spent in the open arms. The 300 mg/kg dose produced more pronounced effects than the 200 mg/kg dose and approached the efficacy of diazepam. These findings indicate that the extract effectively reduced anxiety-like behavior and enhanced exploratory activity in a novel environment.

The anxiolytic activity observed in the present investigation may be attributed to the combined action of flavonoids, phenolic compounds, alkaloids, diterpenes, and sterols present in the extract. Flavonoids such as apigenin, chrysin, and quercetin derivatives reported in medicinal plants have been shown to bind to the benzodiazepine site of the GABA receptor complex, thereby enhancing inhibitory neurotransmission and producing anxiolytic effects similar to benzodiazepines but with fewer adverse effects. Phenolic compounds further contribute by reducing oxidative stress and neuroinflammation, both of which play important roles in the pathogenesis of anxiety disorders. The synergistic interaction among these phytochemicals may therefore explain the significant behavioral improvements observed in the treated animals.

Table 1: Results of percentage yield of extract of *Pergularia daemia*

S. No.	Hydroalcoholic extract	Percentage yield (w/w)
1.	<i>Pergularia daemia</i>	10.5%

Table 2: Result of phytochemical screening of extract of *Pergularia daemia*

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids	
	A) Wagner's Test:	+Ve
	B) Hager's Test:	+Ve
2.	Glycosides	
	A) Legal's Test:	-Ve
3.	Flavonoids	
	A) Lead acetate Test:	+Ve
	B) Alkaline Reagent Test:	+Ve
4.	Saponins	
	A) Froth Test:	-Ve
5.	Phenol	
	A) Ferric Chloride Test:	+Ve
6.	Proteins	
	A) Xanthoproteic Test:	-Ve
7.	Carbohydrate	
	A) Fehling's Test:	+Ve
8.	Diterpenes	
	A) Copper acetate Test:	+Ve
9.	Tannins	
	Gelatin Test:	-ve
10.	Sterols	
	Salkowski Test:	+ve

[+Ve= Positive; -Ve= Negative]

Table 3: Estimation of total phenol, flavonoids and alkaloids content of *Pergularia daemia*

S. No.	Hydroalcoholic extract	Total phenol content	Total flavonoids content mg/100mg	Total alkaloids content
1.	<i>H. longicorniculata</i>	0.67	0.81	0.25

Table 4: Effect of *Pergularia daemia* extract and diazepam in stair case test

Groups	Staircase Test	
	No. of climbing in 3 min	No. of rearing in 3 min
Control (Vehicle 6 ml/kg, p.o)	20.18 ± 1.36	9.24 ± 0.58
<i>H. longicorniculata</i> extract (200 mg/kg, p.o)	13.64 ± 0.84	7.18 ± 0.46
<i>H. longicorniculata</i> extract (300 mg/kg, p.o)	8.26 ± 0.61**	5.41 ± 0.49**
Diazepam (4 mg/kg, i.p.)	4.86 ± 0.49**	3.94 ± 0.42**

All values are Mean ± SEM, n = 6, *P when compared with control.

Table 5: Effect of *Pergularia daemia* extract and diazepam in elevated plus-maze test

Groups	No. of entry into closed arms	No. of entry into open arms	Time spent in open arms (s)
Control (Vehicle 6 ml/kg, p.o.)	12.18 ± 1.24	4.26 ± 0.58	89.64 ± 8.12
<i>H. longicorniculata</i> extract (200 mg/kg, p.o.)	9.24 ± 0.96	6.48 ± 0.72	121.84 ± 10.36*
<i>H. longicorniculata</i> extract (300 mg/kg, p.o.)	6.58 ± 0.84**	8.22 ± 0.81**	157.36 ± 12.18**
Diazepam (4 mg/kg, i.p.)	5.62 ± 0.73**	9.68 ± 0.76**	198.52 ± 14.45**

All values are Mean ± SEM, n = 6, *P when compared with control.

CONCLUSION

The present study demonstrated that the hydroalcoholic extract of *Pergularia daemia* possesses significant phytochemical constituents and promising anxiolytic activity. Preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, phenolic compounds, carbohydrates, diterpenes, and sterols, while quantitative estimation revealed appreciable amounts of total phenolics, flavonoids, and alkaloids. Behavioral evaluation using the staircase test and elevated plus-maze model showed a dose-dependent reduction in anxiety-related behavior, with the 300 mg/kg dose producing greater anxiolytic effects than the 200 mg/kg dose and exhibiting activity comparable to the standard drug diazepam. The observed pharmacological activity may be attributed to the synergistic action of flavonoids, phenolic compounds, alkaloids, and other bioactive constituents present in the extract. These findings scientifically support the traditional use of *Pergularia daemia* as a potential herbal anxiolytic agent and indicate its promise for the development of safe and effective plant-based formulations for the management of anxiety disorders. Further studies involving isolation of active constituents, elucidation of their mechanisms of action, long-term toxicity evaluation, and clinical investigations are recommended to establish its therapeutic potential.

REFERENCE

1. Mendlowicz MV and Stein MB. Quality of life in individuals with anxiety disorders. *Am J Psychiatry*. 2000;157(5):669-682.
2. Naveen V, Felic SV, Aswin A and Arul PK. A review on current understanding, clinical manifestations, and therapeutic approaches of anxiety disorders. *J Pharma Insights Res*. 2024;2(5):173-181.
3. Dar RA, Shahnawaz M, Ahanger MA and Majid IU. Exploring the diverse bioactive compounds from medicinal plants: A review. *J Phytopharm*. 2023;12(3):189-195.
4. Cortese BM and Phan KL. The role of glutamate in anxiety and related disorders. *CNS Spectr*. 2005;10(10):820-830.
5. Chandak RR and Dighe NS. A review on phytochemical and pharmacological profile of *Pergularia daemia* Linn. *J Drug Deliv Ther*. 2019;9(4):809-814.
6. Poddar S, Sarkar T, Choudhury S, Chatterjee S and Ghosh P. Indian traditional medicinal plants: A concise review. *Int J Bot Stud*. 2020;5(5):174-190.
7. Hussein RA and El-Anssary AA. Plants secondary metabolites: The key drivers of the pharmacological actions of medicinal plants. In: Builders PF, editor. *Herbal Medicine*. London: IntechOpen; 2019. p. 11-30.

8. Teleanu DM, Niculescu AG, Lungu II, Radu CI, Vladâcenco O, Roza E, Costăchescu B, et al. An overview of oxidative stress, neuroinflammation, and neurodegenerative diseases. *Int J Mol Sci.* 2022;23(11):5938.
9. Tambun R, Alexander V, Ginting Y. Performance comparison of maceration method, soxhletation method, and microwave-assisted extraction in extracting active compounds from soursop leaves (*Annona muricata*): A review. *IOP Conf Ser Mater Sci Eng.* 2021;1122:012001.
10. Jagessar RC. Phytochemical screening and chromatographic profile of the ethanolic and aqueous extract of *Passiflora edulis* and *Vicia faba* L. (Fabaceae). *J Pharmacogn Phytochem.* 2017;6(6):1714-1721.
11. Kumar R, Sharma S and Devi L. Investigation of total phenolic, flavonoid contents and antioxidant activity from extract of *Azadirachta indica* of Bundelkhand region. *Int J Life Sci Sci Res.* 2018;4(4):1925-1933.
12. Khan MS, Yusufzai SK, Rafatullah M, Sarjadi MS and Razlan M. Determination of total phenolic content, total flavonoid content and antioxidant activity of various organic crude extracts of *Licuala spinosa* leaves from Sabah, Malaysia. *ASM Sci J.* 2018;11(Spec Issue 3):53-58.
13. Prajapati D, Makvana A, Patel M, Chaudhary R and Patel R. Spectrophotometric method for the determination of total alkaloids in selected plant parts. *J Pharmacogn Phytochem.* 2024;13(5):491-494.
14. Kumar A, Singh J and Sharma A. Comparative antianxiety activity evaluation of *Argyreia speciosa* Linn. (roots), *Caesalpinia digyna* Rottler (roots) and *Sphaeranthus indicus* Linn. (flowers). *Int J Pharm Sci Res.* 2015;6(10):4226-4229.
15. Lister RG. Ethologically based animal models of anxiety disorders. *Pharmacol Ther.* 1990;46(3):321-340.