EXTRACTION, PHYTOCHEMICAL SCREENING AND ANTI-ANXIETY ACTIVITY OF *HABENARIA LONGICORNICULATA* EXTRACT

Ambika Lodhi*, Dr. Ritesh Agrawal, Dr. Udit Narain Soni

Oriental College of Pharmacy, Bhopal (MP)

Received: Oct 31, 2025 Accepted: Nov 20, 2025 Published: Nov 27, 2025

Abstract

The present study aimed to evaluate the phytochemical composition and anxiolytic antidepressant potential of the hydroalcoholic extract of *Habenaria longicorniculata*. The plant extract was prepared using a hydroalcoholic solvent system, yielding 13.5% w/w. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, carbohydrates, and diterpenes, while glycosides, saponins, and proteins were absent. Quantitative estimations confirmed appreciable levels of total flavonoids (0.968 mg/100 mg), total phenols (0.532 mg/100 mg), and total alkaloids (0.147 mg/100 mg). The anxiolytic activity was assessed using the Staircase Test and Elevated Plus-Maze (EPM) test, while antidepressant effects were evaluated using the Tail Suspension Test (TST) and Forced Swim Test (FST). The extract at 300 mg/kg produced a significant reduction in climbing and rearing behavior in the Staircase Test and a marked increase in time spent in open arms in the EPM, comparable to diazepam. Furthermore, the extract demonstrated significant antidepressant-like effects, reducing immobility in both TST and FST in a dose-dependent manner. These findings suggest that *Habenaria longicorniculata* possesses notable anxiolytic and antidepressant properties, likely attributed to its rich flavonoid, phenolic, and alkaloid content. The study provides scientific support for the traditional use of the plant and highlights its potential as a natural therapeutic agent for managing anxiety and depressive disorders.

Keywords: *Habenaria longicorniculata*, Hydroalcoholic extract, Phytochemical screening, Anxiolytic activity, Antidepressant activity, Elevated plus-maze, Tail suspension test, Forced swim test, Flavonoids, Alkaloids.

Introduction

Anxiety disorders are among the most prevalent neuropsychiatric conditions globally, affecting nearly 301 million individuals and significantly impairing quality of life, work productivity, and social functioning (Pirkola et al., 2023). Current pharmacological treatments including

benzodiazepines, selective serotonin reuptake inhibitors (SSRIs), and serotonin norepinephrine reuptake inhibitors (SNRIs) are effective but associated with several limitations such as tolerance, dependence, cognitive impairment, and withdrawal symptoms (Dell'Osso et al., 2010). These drawbacks highlight the urgent need for alternative therapeutic agents that are both safe and effective. In recent years, medicinal plants have gained considerable attention as potential sources of anxiolytic compounds due to their rich phytochemical diversity and favorable safety profiles (Balkrishna et al., 2025).

The genus Habenaria, belonging to the family Orchidaceae, comprises more than 800 species distributed across tropical and subtropical regions. Several species of *Habenaria* are used in traditional Ayurvedic and folk medicine for treating nervous disorders, inflammation, fatigue, and general debility (Pedron et al., 2014). *Habenaria longicorniculata*, commonly known as "Long-spurred Habenaria," is an epiphytic orchid widely distributed in the Western Ghats of India. It has been traditionally used as a rejuvenating tonic and is believed to enhance vitality, memory, and stress tolerance (Zhang et al., 2014). Despite its ethnomedicinal relevance, scientific validation of its neuropharmacological activities including anxiolytic effects remains largely unexplored.

Preliminary phytochemical investigations of Habenaria species have reported the presence of alkaloids, flavonoids, phenols, terpenoids, tannins, and glycosides (Arora et al., 2023).

These classes of bioactive compounds are known to modulate neurotransmitter pathways such as GABAergic, serotonergic, and oxidative stress mechanisms, which play crucial roles in anxiety regulation (Ghallab and Elassal; 2024). Therefore, systematic extraction and phytochemical screening of *H. longicorniculata* may help identify potential anxiolytic constituents responsible for its traditional uses.

Given the global interest in plant-based anxiolytics and the lack of comprehensive studies on this orchid, the present investigation was undertaken to evaluate the extraction, phytochemical profile, and anti-anxiety activity of *Habenaria longicorniculata* extract using validated animal models. This study aims to provide scientific evidence supporting the therapeutic potential of this medicinally important orchid.

Material and Methods

Material

A variety of high-quality chemicals and analytical reagents were used in the study to maintain accuracy and reliability. Potassium mercuric iodide, picric acid, and ferric chloride were obtained from Thomas Baker, while Loba Chemie supplied iodine, potassium iodide, sodium nitroprusside, sodium hydroxide, lead acetate, and Folin–Ciocalteu reagent. Pyridine, nitric acid, copper acetate, and potassium bismuth iodide were procured from S.D. Fine Chem. Ltd., and solvents such as methanol, ethanol, and chloroform were purchased from Qualigens Fine Chemicals. Fehling's solution was sourced from CDH, New Delhi, and standards like quercetin and gallic acid from HiMedia. The instrumentation included a Labindia UV-visible spectrophotometer (3000+), REMI microcentrifuge, Accumax pH meter, Contech electronic balance, Oracle hot air oven, Ambros vortex mixer, Microtech rotary vacuum evaporator, and a sonicator from Athena Technology, all of which ensured precision in phytochemical screening and quantitative analysis.

Methods

Collection of plant materials

Every parts of the plant like bark, leaves, flowers, roots, fruits and seeds may contain active secondary metabolites. Fresh and healthy aerial parts of *Habenaria longicorniculata* were collected from ruler area of Bhopal (M.P.) in the month of March, 2025.

Extraction by maceration process

50 gram of shade dried aerial parts of *Habenaria longicorniculata* 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 *Habenaria longicorniculata* 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 (Kulkarni, 2025).

Determination of percentage yield

The extraction yield is evaluate of the solvent's efficiency to extracts bioactive components from the selected natural plant samples and it was defined as quantity of plant extracts recovered in mass after solvent extraction compared with the initial quantity of plant samples. 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:

$$\textbf{Percentage Yield} = \frac{Weight\ of\ Extract}{Weight\ of\ Powder\ drug\ taken} x\ 100$$

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, terpenoids, phenols, flavonoids etc.). Phytochemical examinations were carried out for all the extracts as per the standard methods (Jagessar *et al.,* 2017).

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 (Kumar *et al.,* 2018).

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method. 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% $AlCl_3$ 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 (Khan *et al.,* 2018).

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. 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 (Prajapati *et al.,* 2024). 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 *Habenaria longicorniculata* 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 *Habenaria longicorniculata* 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.

Animals

The animals were maintained in colony cages at 25 ± 2◦C, relative humidity 50–55% maintained under 12 h light and dark cycle (6–10 h light, 18–6 h dark). The animals were fed with Standard animal feed (Hindustan Lever Ltd.) and water was applied ad libitum. All the animals were acclimatized to the laboratory conditions prior to experimentation. Experimental protocol was approved by Institutional Animal Ethics Committee. Care of the animals was taken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment Forests and climate change, Government of India. Experiment protocol was approved by Institutional Animal Ethics Committee. The anti-anxiety activity was evaluated using staircase test and elevated plus maize test.

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 *Habenaria longicorniculata* (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.

Table 1: Dosing and grouping of animals

S. No	Groups	Dose
Group-II	Control	Vehicle 6 ml/kg, p.o
Group-III	Treated with hydroalcoholic extract of *Habenaria longicorniculata*	200 mg/kg, p.o
Group-IV	Treated with hydroalcoholic extract of *Habenaria longicorniculata*	300 mg/kg, p.o
Group-V	Treated with (Std) Diazepam	4 mg/kg, i.p

The anti-anxiety activity was evaluated using staircase test and elevated plus maize test.

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 (Kumar *et al.,* 2015).

Elevated plus maze

The apparatus consist of two open arms (5 × 10 cm) and two closed arms (5 × 10 × l5 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 (Lister, 1990). 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.

Tail Suspension Test

The tail-suspension test is a mouse behavioural test that is useful for screening possible antidepressant medications and testing other manipulations that are likely to influence behaviours associated with depression. Mice are suspended with tape by their tails, in such a way that they can not escape or hang on to surfaces nearby. The resulting escape oriented behaviours are quantified during this test, usually six minutes in length. The tail-suspension test for high-throughput screening of prospective antidepressant compounds is a valuable method for drug discovery. Here, with additional focus on possible issues that can arise and how to prevent them, we explain the information needed for the implementation of this test. The followings are group distribution,

Group I (Normal Control): Received normal saline.

Group II (Standard Control): Received Diazepam.

Group III (Test Group–I): Treated with hydroalcoholic extract of *Habenaria longicorniculata* at a dose of 200 mg/kg, p.o.

Group 1V (Test Group–II): Treated with hydroalcoholic extract of *Habenaria longicorniculata* at a dose of 300 mg/kg, p.o.

The tail suspension method used in this study was similar to those describe by Steru *et al.,* (1985). Treatment was given 60 min prior to study as described by study design. Mice were suspended on the edge of the table, 50 cm above the floor, with the help of adhesive tape placed approximately 1 cm from the tip of the tail. The total duration of immobility induced by tail suspension was recorded during a 6 min of the 10 min period. Animal was considered to be immobile when it did not show any movement of the body, hanged passively and completely motionless (Steru *et al.,* 1985).

Forced Swim Test

The forced swim test is a rodent behavioural test used to evaluate antidepressant drugs, new compounds' antidepressant efficacy, and experimental manipulations aimed at rendering depressive-like states or preventing them. Mice are placed in an inescapable transparent tank filled with water and their mobility activity related to escape is measured. The forced swim test is straightforward and requires minimal specialised equipment to perform reliably. Successful implementation of the forced swim test requires certain procedural details to be adhered to and

unjustified stress on the mice to be minimised. In the overview of the protocol and the accompanying video, we explain how to execute this test's mouse version with focus on possible pitfalls that may be harmful to the interpretation of results and how to prevent them. The following are the distribution of groups,

Group I: Normal received normal saline

Group II: Received Diazepam as a standard control

Group III: Received hydroalcoholic extract of *Habenaria longicorniculata* -200 mg/kg, p.o.

Group IV: Received hydroalcoholic extract of *Habenaria longicorniculata* -300 mg/kg, p.o.

Mice were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm) containing 19 cm of water at 25±1°C for the forced swimming test (FST). As described in the study design, treatment was given 60 minutes prior to the study. Both animals were required to swim for 6 minutes and the immobility period was observed and assessed during the final 4-minute test interval. When it ceased to struggle and remained floating motionless in the water, each mouse was judged to be immobile, making only certain movements to keep its head above water. A decrease in immobility duration is indicative of an antidepressant-like effect (Porsolt *et al.,* 1977).

Statistical analysis

Results were expressed as Mean ± 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 hydroalcoholic extraction of *Habenaria longicorniculata* yielded 13.5% w/w, indicating efficient solubilization of its phytoconstituents (Table 1). Preliminary phytochemical analysis revealed the presence of alkaloids, flavonoids, phenols, carbohydrates, and diterpenes, whereas glycosides, saponins, and proteins were absent (Table 2). The presence of these bioactive secondary metabolites especially alkaloids and flavonoids is pharmacologically significant because such compounds are well known for their CNS-modulating properties, including anxiolytic and antidepressant activity.

Quantitative analysis (Table 3) confirmed that the extract contains notable amounts of total flavonoids (0.968 mg/100 mg) and total phenols (0.532 mg/100 mg), along with a moderate level of alkaloids (0.147 mg/100 mg). These compounds are frequently associated with

neuroprotective actions, modulation of GABAergic transmission, antioxidant activity, and suppression of stress-induced neurochemical changes. Thus, their presence provides a biochemical basis for the observed CNS activity in animal models.

The staircase test is sensitive to anxiolytic agents, which reduce the number of climbing and rearing events. The extract at 200 mg/kg produced a mild reduction, while the 300 mg/kg dose produced a significant decrease in climbing and rearing ($p < 0.01$), approaching the standard diazepam (Table 4). This reduction reflects decreased exploratory behavior due to anxiolytic action rather than sedation.

In the EPM test, anxiolytic compounds increase open-arm exploration and time spent in open arms. The 300 mg/kg dose significantly increased open-arm time (149.28 ± 10.12 s, $p < 0.05$) compared to control, while diazepam showed the highest activity (196.45 ± 15.26 s, $p < 0.01$). The reduction in closed-arm entries further supports an anxiolytic effect of the extract. These findings suggest that *H. longicorniculata* enhances risk-taking or reduces anxiety-like behavior, similar to benzodiazepine-like activity.

In the TST (Table 5), the extract produced a significant reduction in immobility duration, with 22.21% inhibition (200 mg/kg) and 26.89% inhibition (300 mg/kg), compared to 39.36% inhibition by diazepam. Decreased immobility reflects antidepressant-like activity, possibly mediated through monoaminergic modulation or reduction of behavioral despair.

A similar trend was observed in the FST (Table 6). The extract reduced immobility duration by 33.83% (200 mg/kg) and 41.85% (300 mg/kg), indicating notable antidepressant-like effects, though slightly lower than diazepam (49.82%). Consistency between TST and FST confirms the extract's antidepressant potential.

The combined results of phytochemical content and pharmacological evaluation indicate that *Habenaria longicorniculata* extract possesses significant anxiolytic and antidepressant activities. The presence of alkaloids, flavonoids, and phenols likely contributes to these effects through modulation of GABAergic, serotonergic, or antioxidant pathways. The 300 mg/kg dose consistently exhibited stronger CNS activity, approaching the efficacy of the standard drug diazepam without causing excessive sedation. These findings support the traditional use of this plant and highlight its potential as a natural therapeutic agent for anxiety and depressive disorders.

Table 1: Results of percentage yield of extract of *Habenaria longicorniculata*

S. No.	Hydroalcoholic extract	Percentage yield (w/w)
1.	*Habenaria longicorniculata*	13.5%

Table 2: Result of phytochemical screening of extract of *Habenaria longicorniculata*

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

[+Ve= Positive; -Ve= Negative]

Table 3: Estimation of total phenol, flavonoids and alkaloids content of *Habenaria longicorniculata*

S. No.	Hydroalcoholic extract	Total phenol content	Total flavonoids content	Total alkaloids content
		mg/100mg		
1.	*H. longicorniculata*	0.532	0.968	0.147

Table 4: Effect of *Habenaria longicorniculata* extract and diazepam in stair case test and elevated plus-maze test

Groups	Staircase Test		Elevated Plus-Maze Test		
	No. of climbing in 3 min	No. of rearing in 3 min	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)	19.35 ± 1.42	8.91 ± 0.63	12.28 ± 1.18	8.79 ± 1.36	92.4 ± 7.68
H. longicorniculata extract (200 mg/kg, p.o)	12.42 ± 0.78	7.28 ± 0.48	8.95 ± 1.28	5.81 ± 0.85	108.32 ± 11.05
H. longicorniculata extract (300 mg/kg, p.o)	7.32 ± 0.58**	5.12 ± 0.53**	6.51 ± 1.15**	5.96 ± 1.58	149.28 ± 10.12*
Diazepam (4 mg/kg, i.p)	5.18 ± 0.62**	4.22 ± 0.57**	6.05 ± 0.93**	3.72 ± 1.29*	196.45 ± 15.26**

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

Table 5: Effect of *Habenaria longicorniculata* extract on TST in mice

Sr. No.	Groups	No. of Animals	Dose (mg/kg or mL/kg, p.o./i.p.)	Duration of Immobility (sec) (Mean ± SD)	% Change in Immobility Duration
1	Normal Control	06	5 mL/kg, p.o.	148.5 ± 5.60	–
2	Diazepam (Standard)	06	4 mg/kg, i.p.	90.0 ± 4.12 ***	39.36
3	*Habenaria longicorniculata* Extract (200 mg/kg)	06	200 mg/kg, p.o.	115.5 ± 3.78	22.21
4	*Habenaria longicorniculata* Extract (300 mg/kg)	06	300 mg/kg, p.o.	108.6 ± 4.21	26.89

Table 6: Effect of *Habenaria longicorniculata* extract on FST in mice

Sr. No.	Groups	No. of Animals	Dose (mg/kg or mL/kg, p.o./i.p.)	Duration of Immobility (sec) (Mean ± SD)	% Change in Immobility Duration
1	Normal Control	06	5 mL/kg, p.o.	140.33 ± 6.60	–
3	Diazepam (Standard)	06	4 mg/kg, i.p.	70.40 ± 5.82*	49.82
4	*Habenaria longicorniculata* Extract (200 mg/kg)	06	200 mg/kg, p.o.	92.85 ± 4.95	33.83
5	*Habenaria longicorniculata* Extract (300 mg/kg)	06	300 mg/kg, p.o.	81.60 ± 5.40	41.85

Values are expressed as mean±S.E.M. (n = 6). Values are statistically significant at***P<0.001, **P<0.01, *P<0.05 vs. control group respectively (One-way ANOVA followed by Tukey's post hoc test).

Conclusion

The hydroalcoholic extract of *Habenaria longicorniculata* showed significant anxiolytic and antidepressant activities in experimental models. The presence of flavonoids, alkaloids, phenols, and diterpenes may be responsible for these effects. The extract, especially at 300 mg/kg, effectively reduced anxiety and immobility behaviors, comparable to diazepam. These findings support the traditional use of the plant for anxiety-related disorders and suggest its potential as a natural therapeutic agent.

References

- Pirkola S, Fröjd S, Nurmela K. Depressive and anxiety disorders. *The WASP Textbook on Social Psychiatry: Historical, Developmental, Cultural, and Clinical Perspectives.* 2023:304.
- Dell'Osso B, Buoli M, Baldwin DS, Altamura AC. Serotonin norepinephrine reuptake inhibitors (SNRIs) in anxiety disorders: a comprehensive review of their clinical efficacy. *Human Psychopharmacology: Clinical and Experimental.* 2010 Jan;25(1):17-29.
- Balkrishna A, Agarwal U, Arya D, Chaudhary S, Arya V. From tradition to evidence: exploring the neurochemical basis of medicinal plants in anxiety therapy. *The World Journal of Biological Psychiatry.* 2025 Nov 17;26(8):371-408.
- Pedron M, Buzatto CR, Ramalho AJ, Carvalho BM, Radins JA, Singer RB, Batista JA. Molecular phylogenetics and taxonomic revision of *Habenaria* section *Pentadactylae* (Orchidaceae, Orchidinae). *Botanical Journal of the Linnean Society.* 2014 May 1;175(1):47-73.
- Zhang HP, Wen SJ, Wang H, Ren ZX. Floral nectar reabsorption and a sugar concentration gradient in two long-spurred *Habenaria* species (Orchidaceae). *BMC Plant Biology.* 2023 Jun 22;23(1):331.
- Arora M, Arora K, Kaur R. Pharmacognostic, physicochemical, phytochemical, nutraceutical evaluation and in vitro antioxidant potency of *Habenaria intermedia* D. Don—a rare orchid. *South African Journal of Botany.* 2023 Jan 1;152:278-87.
- Ghallab YK, Elassal OS. Biochemical and neuropharmacology of psychiatric disorders. In: *Nutrition and Psychiatric Disorders: An Evidence-Based Approach to Understanding the Diet-Brain Connection.* Singapore: Springer Nature Singapore; 2024 Jun 29. p. 25–47.

- Kulkarni BL, Wadulkar RD, Satpute KL, Khare MP, Survase SS. A review on anti-anxiety plants. *International Journal of Pharmaceutical Sciences.* 2025;3(6):3879–3890.
- Jagessar RC. Phytochemical screening and chromatographic profile of the ethanolic and aqueous extract of *Passiflora edulis* and *Vicia faba* L. (Fabaceae). *Journal of Pharmacognosy and Phytochemistry.* 2017;6(6):1714–1721.
- Kumar R, Sharma S, Devi L. Investigation of total phenolic, flavonoid contents and antioxidant activity from extract of *Azadirachta indica* of Bundelkhand region. *International Journal of Life Sciences and Scientific Research.* 2018;4(4):1925–1933.
- Khan A, Akram M, Thiruvengadam M, Daniyal M, Zakki SA, Munir N, et al. Anti-anxiety properties of selected medicinal plants. *Current Pharmaceutical Biotechnology.* 2022 Jul 1;23(8):1041–1060.
- Digna Prajapati, Ami Makvana, Mohini Patel, Ranna Chaudhary, Ruby Patel. Spectrophotometric method for the determination of total alkaloids in selected plant parts. *Journal of Pharmacognosy and Phytochemistry.* 2024;13(5):491–494.
- Elayaraja A, Rahaman SA, Kumar P, Kumar P. Anti-anxiety activity of hydroalcoholic extract of *Scoparia dulcis* Linn. assessed using different experimental anxiety models in rodents. *International Journal of Pharmacological Research.* 2015 Jan 1;5(3):62–67.
- Lister RG. Ethologically based animal models of anxiety disorders. *Pharmacology and Therapeutics.* 1990;46:321–340.
- Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology.* 1985;85:367–370.
- Porsolt R, Bertin A, Jalfre M. Behavioral despair in mice: a primary screening test for antidepressants. *Archives Internationales de Pharmacodynamie et de Therapie.* 1977;229(2):327–336.