

Evaluation Of Antianxiety Profile Of *Alternanthera Pungens* Aerial Parts

Noel Mankoo¹, Amandeep Kaur Gill^{2*}, Monika Gupta³, Ajay Singh Kushwah⁴

¹Research Scholar, School of Pharmaceutical Sciences, CT University, Ludhiana-142024, Punjab, India.

Email: noelchemistry@gmail.com

²Associate Professor, School of Pharmaceutical Sciences, CT University, Ludhiana-142024, Punjab, India.

Email: amansatnamgill@gmail.com

³Professor, Head of Department, Amar Shaheed Baba Ajit Singh Jujhar Singh Memorial College of Pharmacy, Bela, Punjab. Email: monikaguptaa@gmail.com

⁴Professor & Head, Department of Pharmacology, Amar Shaheed Baba Ajit Singh Jujhar Singh Memorial College of Pharmacy, Bela 140111 Ropar, Punjab, India (An Autonomous College).

Email: kushwah_ph05@yahoo.co.in; Institutional Email: ajay@copbela.org; ORCID: 0000-0003-0559-7670

*Corresponding Author: Dr. Amandeep Kaur Gill, Associate Professor, School of Pharmaceutical Sciences, CT University, Ludhiana-142024, Punjab, India. Email: amansatnamgill@gmail.com

ABSTRACT

Though the plant has not been thoroughly studied to support its traditional claims, *Alternanthera pungens* (Kantevalisanti; Amaranthaceae) has been used traditionally to treat a variety of illnesses, primarily anxiety. Therefore, it was intended to use the elevated plus maze model (EPM) to examine the antianxiety properties of different extracts and fractions of *Alternanthera pungens* aerial parts. The aerial components of *Alternanthera pungens* that were correctly identified were extracted in a methodical and thorough manner utilizing solvents in ascending order of polarity, namely n-hexane, chloroform, methanol, and water using Soxhlet technology. The antianxiety effects of all phytochemical-rich crude extracts were tested in mice at dosages of 100, 200, or 400 mg/kg, p.o. *Alternanthera pungens*' effectiveness was statistically contrasted with that of diazepam, a common anxiolytic medication (2 mg/kg, i.p.). At a dose of 400 mg/kg, the methanol extract was statistically equal to the standard medication and showed considerable antianxiety action when compared to the control. There was some moderate antianxiety action in the chloroform extract. n-hexane, ethyl acetate, and 1-butanol were the solvents used to partition the bioactive methanol extract in ascending order of polarity. All fractions rich in phytochemicals were tested for antianxiety effects in mice at dosages of 25 or 50 mg/kg, p.o. At a dose of 50 mg/kg, only the ethyl acetate fraction demonstrated considerable antianxiety effect comparable to the standard medication. Current research has scientifically confirmed *Alternanthera pungens*' traditional claims of antianxiety properties. It has been proposed that phenolic and flavonoid chemicals found in the aerial sections of *Alternanthera pungens* may help alleviate anxiety problems.

Keywords: *Alternanthera pungens*, Anxiolytic, Diazepam, Elevated plus maze, Flavonoids.

How to cite this article: Mankoo N, Gill AK, Gupta M, Kushwah AS. Evaluation of Antianxiety Profile of *Alternanthera pungens* Aerial Parts. Int J Drug Deliv Technol. 2026;16(12s): 715-720. DOI: 10.25258/ijddt.16.12s.85

INTRODUCTION

Anxiety is defined as a central nervous system disorder in which a patient suffers from an emotional state of mind, discomfort, uneasiness and fear about future (Tripathi, 1999). It is thus explained as mental disorder in which pathological anxiety causes disturbance of mood as well as affects thinking, behaviour and physiological activity (Adwas *et al*, 2019; Munir *et al*, 2019).

Various disorders are associated with anxiety like panic disorder, agoraphobia, generalized anxiety disorder, specific phobia, social phobia, obsessive-

compulsive disorder, acute stress disorder, and post-traumatic stress disorder. In addition, there are adjustment disorders with anxiety features, and disorders due to general medical conditions and substance-induced anxiety disorders (Munir *et al*, 2019; Greenberg *et al*, 1999).

The etiology of anxiety includes stress, diabetes, depression, genes, environmental factors such as either child or substance use (Munir *et al*, 2019).

Anxiety symptoms and the resulting disorders are thought to be due to disrupted modulation within the central nervous system. Physical and emotional

manifestations of this dysregulation are the result of heightened sympathetic arousal of varying degrees (Kaplan *et al.*, 1995). Although some anxiety is a part of normal life but when it affects an emotional state of person, then to combat the symptoms, anti-anxiety drugs are required. Although there are many classes of synthetic Antipsychotic drugs which are used to cure anxiety but due to side effects, there is an urge to look into natural substances which can cure the underlying cause of anxiety. One such plant *viz.*, *Alternanthera pungens* has been chosen to explore its anti-anxiety activity.

It is a native of central and South America but is also reported from other tropical countries including India (Wild, 1995). It is a perennial herb and commonly noticed as a mat like structure in vacant lots, along roadside, railway tracks, lawns, etc. Its stem is hairy, 10-50 cm long, prostrate and occasionally develop roots the nodes. Leaves are green and ovate to obovate in shape and are generally 0.5 to 4.5 cm long and 0.3 to 2 cm wide. Flowers are without stalk, sparsely velvety spikes with spiny bracts and bracteoles (Naidu, 2012). In traditional medicine it was used as painkiller, for stomach ache, swelling and nasopharyngeal infections and also reported for lactation stimulus in veterinary (Burkill, 1985). The phytoconstituents present in the plant are saponins, alkaloids, steroids, triterpenoids, flavanoids, anthraquinones, tannins, cardiac glycosides, coumarins, leucoanthocyanins (Alekhya *et al.*, 2021). The review of literature suggested that phytopharmacological investigations related to antianxiety activity guided fractionation has never been done on this potential plant till date. Thus, the present research work will be planned to isolated anxiolytic compounds by bioactivity-directed-fractionation using sophisticated chromatographic technologies.

MATERIALS AND METHODS

Collection and identification of plant material

In April 2024, aerial pieces of *Alternanthera pungens* were gathered at National Institute of Pharmaceutical Education and Research (NIPER). As per the reference number NIP-H-3127 dated 03/09/2024, Dr. Alok Goyal, Chief Scientist and Head of the Raw Materials Herbarium and Museum at the Mohali verified the identity of the plants.

Solvents

A variety of crude extracts and fractions of *Alternanthera pungens* aerial parts were prepared using methanol (S.D. Fine Chemicals, Mumbai,

India), chloroform, n-hexane, 1-butanol, and ethyl acetate (E Merck, Delhi, India) of LR grade instead.

Preparation of extracts and fractions

A. pungens aerial components were used to create a variety of crude, uncharacterized extracts, including n-hexane extract (HE), chloroform extract (CE), methanol extract (ME), and water extract (WE). Figure 2 showed the methodical process of preparing several uncharacterized crude extracts.

A round-bottom flask containing 500 g of the plant material's bioactive extract was filled with 1 L of water, and the mixture was divided into three parts using 200 ml of solvents arranged in increasing polarity order: n-hexane, ethyl acetate, and 1-butanol. The mixture was heated to 50°C for 30 minutes while being constantly stirred. Eight more times, this partitioning process was carried out using each solvent. The solvent's separated layers were combined and condensed under low pressure to obtain the 1-butanol fraction (BF), n-hexane fraction (HF), ethyl acetate fraction (EAF), and residual bioactive extract (RBE). Preliminary phytochemical tests (Farnsworth, 1966) and antianxiety activity in mice using the elevated plus maze paradigm (EPM) were conducted on a variety of plant extracts and fractions (Kulkarni, 2003).

Animals

The Central Research Institute in Kasauli, India provided Swiss-Albino mice (either sex) weighing 20–25 g as the animals used for the acute toxicity and antianxiety activity tests. The animals were given an unlimited supply of water and a standard laboratory pellet meal. Prior to conducting animal studies, permission was obtained from the Institutional Animal Ethics Committee, (IAEC) of ASBASJSM College of Pharmacy, Bela (Ropar), Punjab with approval number ASCB/IAEC/19/24/20. Before the trial began, the animals were continuously acclimated to laboratory surroundings for one hour every day for seven days. In accordance with the Committee for the Purpose of Control and Supervision on Experiments on Animals' rules, all of the experiments were conducted between the hours of 9 AM and 12 PM. Every set of trials used groups of six animals. Before use, the animals were fasted for the whole night. Using a tuberculin syringe equipped with an oral cannula, the test medication dosages were given orally. Vehicle and standard drug

The vehicle for creating different test doses of the extracts, fractions, and isolates of both plants

Evaluation of Antianxiety profile of *Alternanthera pungens* Aerial Parts

was distilled water plus 2 percent Tween 80. The typical medication used to treat anxiety was diazepam.

Experimental design for the assessment of antianxiety activity

Two experimental protocols were designed. Forty eight groups of animals were made, and each group comprised 5 animals.

Experimental protocol I, comprising 8 groups, was designed to assess antianxiety activity of various crude extracts of *A. pindrow* aerial parts.

Group 1 - Control group received vehicle (0.25 ml, *p.o.*).

Group 2 - Standard group received diazepam (2 mg/kg, *i.p.*).

Groups 3, 4 and 5 - Test groups received 100, 200 and 400 mg/kg doses of CE respectively.

Groups 6, 7 and 8 - Test groups received 100, 200 and 400 mg/kg doses of ME respectively.

Experimental protocol II, comprising 6 groups, was designed to assess antianxiety activity of various fractions obtained from bioactive extract of *A. pungens* aerial parts.

Group 1 - Control group received vehicle (0.25 ml, *p.o.*).

Group 2 - Standard group received diazepam (2 mg/kg, *i.p.*).

Groups 3 and 4 - Test groups received 25 and 50 mg/kg doses of EAF respectively.

Groups 5 and 6 - Test groups received 25 and 50 mg/kg doses of BF respectively.

Elevated plus maze model

Two open arms (16 x 5 cm) and x 5 x 12 cm) with an open can d maze device (Kulkarni, 2003) raised from the ground by 25 c minutes after the treatments, positioned with its head facing center of the elevated plus maze. the mouse entered the open ar minute trial, as well as the avera spent there (average time = arms/number of entries in arms),

Statistics

The findings are presented in the form of mean \pm standard deviation (SD). One-way analysis of variance (ANOVA) was used to compare the test medications with the standard drug and control, and Student-Newman-Keul's test was then performed (Scheffer, 1980).

RESULTS AND DISCUSSION

The percentage (w/w) yield of several plant extracts, including n-hexane extract (HE), chloroform extract (CE), methanol extract (ME), and water extract (WE) were found to be 1.70, 1.45, 5.89 and 6.90% w/w, respectively. The fundamental phytochemical screening indicating the presence of fixed oils/lipids in HE; steroids, triterpenoids in CE; cardiac glycosides, flavonoids, tannins, saponins, triterpenoids in ME and carbohydrates, proteins in WE.

The primary phytochemical screening results of several extracts verified that bioactive classes of phytoconstituents were only present in CE and ME. Consequently, mice were used to test the antianxiety activity of only the CE and ME extracts of *A. pungens* aerial parts using EPM. The mean number of entries and mean amount of time that mice spent in the open arms of the EPM following the administration of dosages of crude extracts at 100, 200, or 400 mg/kg, diazepam at 2 mg/kg, and vehicle is displayed in Table 1 and Figure 1. At 100 mg/kg, the CE showed no evidence of antianxiety action; however, in a dose-dependent manner, the CE showed mild antianxiety activity as compared to the control at 200 or 400 mg/kg. Although mice given 100, 200, or 400 mg/kg of ME showed a substantial increase in the number of entrances and time spent in open arms when compared to the control group, only the 400 mg/kg dose of ME showed statistically similar anxiolytic efficacy as per standard drug.

Table 1: Antianxiety activity of CE and ME of *A. pungens* aerial parts using EPM.

| Treatment | Dose (mg/kg) | Number of entries in open arms \pm S.D. | Time spent in open arms (sec) \pm S.D. |
|-----------|--------------|---|--|
| Control | Vehicle | 2.60 \pm 0.54 ^a | 4.60 \pm 0.89 ^a |
| Diazepam | 2 | 11.20 \pm 1.30* | 13.40 \pm 0.88* |
| CE | 100 | 3.60 \pm 0.89 ^a | 5.40 \pm 0.83 ^a |
| | 200 | 4.60 \pm 0.54 ^a | 7.00 \pm 0.70 ^a |
| | 400 | 5.20 \pm 0.83 ^a | 7.20 \pm 0.83 ^a |
| ME | 100 | 5.80 \pm 0.84 ^a | 8.20 \pm 0.85 ^a |
| | 200 | 7.40 \pm 1.14 ^a | 10.00 \pm 0.72 ^a |
| | 400 | 10.80 \pm 1.09* | 12.80 \pm 0.87* |

n=5; The data is expressed as Mean \pm S.D.; * $P < 0.05$ vs Control; ^a $P < 0.05$ vs Diazepam (Standard drug); one way ANOVA followed by Student-Newman-Keul's test.

Evaluation of Antianxiety profile of *Alternanthera pungens* Aerial Parts

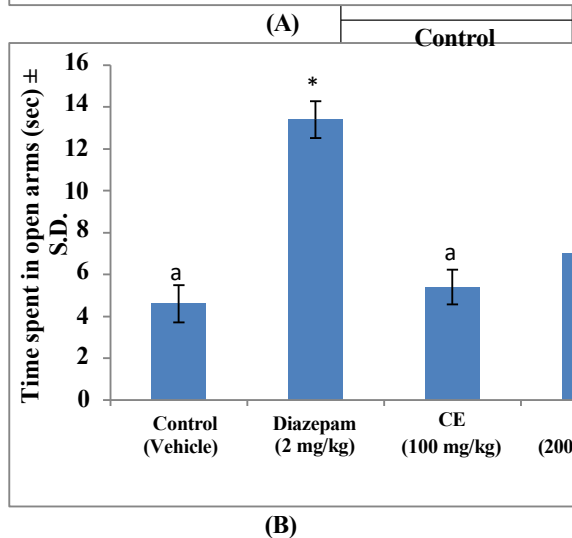
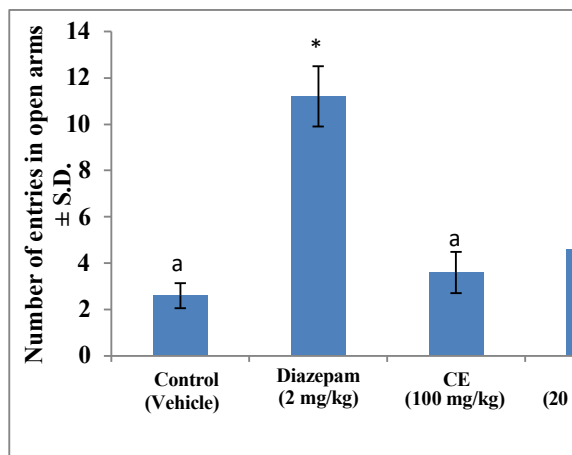


Figure 1: Antianxiety activity of many uncharacterized crude extracts of *A. pungens* aerial parts using EPM. (A) Number of entries in open arms ± S.D. and (B) Time spent in open arms (sec) ± S.D.

The percentage yields (% w/w) of the different fractions extracted from the bioactive methanol extract, including n-hexane, ethyl acetate, 1-butanol, and the remaining bioactive extract were found to be 8.90, 19.58, 26.36 and 39.10 % w/w, respectively, in relation to methanol extract.

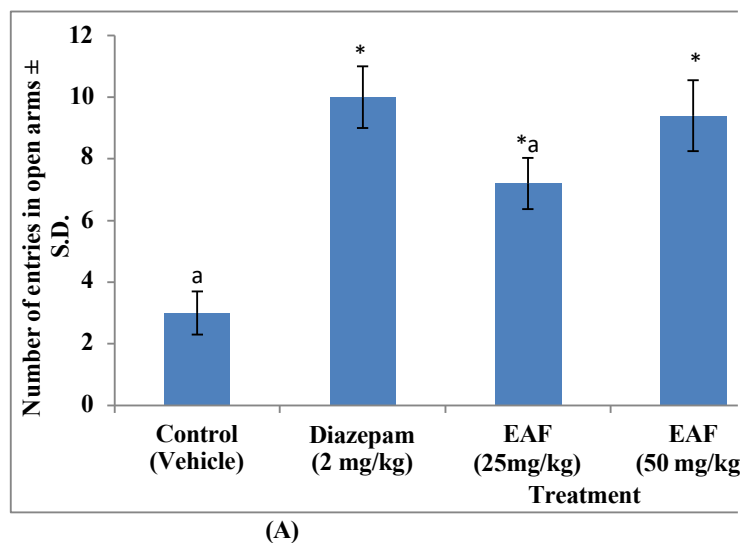
The primary phytochemical screening results of several fractions obtained from bioactive methanol extract of plant aerial parts verified that bioactive classes of phytoconstituents were only present in EAF and BF. Consequently, mice were used to test the antianxiety activity of only the EAF and BF obtained from bioactive methanol extract of plant aerial parts using EPM. The mean number of entries and mean amount of time that mice spent in the open arms of the EPM following the administration of dosages of EAF at 25 or 50 mg/kg, BF at 25 or 50 mg/kg,

diazepam at 2 mg/kg, and vehicle is displayed in Table 2 and Figure 2. BF showed mild antianxiety action in a dose-dependent manner as compared to the control at the 25 or 50 mg/kg. Although mice given 25 or 50 mg/kg of EAF showed a substantial increase in the number of entrances and time spent in open arms when compared to the control group, only the 50 mg/kg dose of EAFE showed statistically similar anxiolytic efficacy as per standard drug.

Table 2: Antianxiety activity of many fractions derived from aerial parts of bioactive methanol extract of *A. pungens* using EPM.

| Dose (mg/kg) | Number of entries in open arms ± S.D. | Time spent in open arms (sec) ± S.D. |
|--------------|---------------------------------------|--------------------------------------|
| Vehicle | 3.00 ± 0.70 ^a | 5.00 ± 0.70 ^a |
| 2 | 10.00 ± 1.00 [*] | 13.60 ± 0.89 [*] |
| 25 | 7.20 ± 0.83 ^{*a} | 9.80 ± 0.83 ^{*a} |
| 50 | 9.40 ± 1.15 [*] | 13.40 ± 1.14 [*] |
| 25 | 4.40 ± 0.55 ^{*a} | 6.00 ± 0.70 ^{*a} |
| 50 | 5.20 ± 0.86 ^{*a} | 7.20 ± 0.85 ^{*a} |

n=5; The data is expressed as Mean ± S.D.; * $P < 0.05$ vs Control; ^a $P < 0.05$ vs Diazepam (Standard drug); one way ANOVA followed by Student-Newman-Keul's test.



Evaluation of Antianxiety profile of *Alternanthera pungens* Aerial Parts

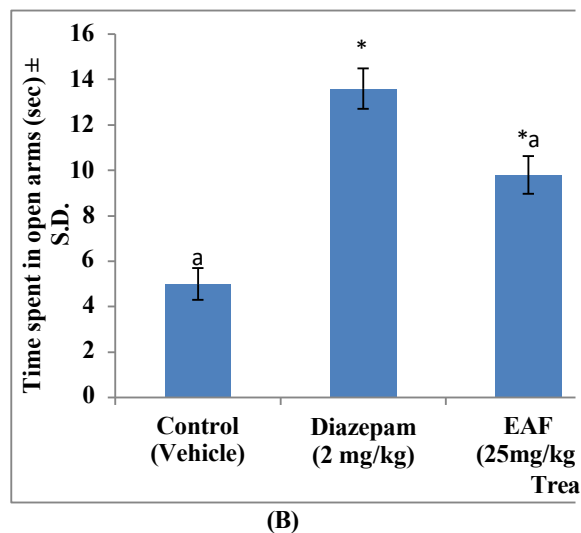


Figure 2: Antianxiety activity of many fractions derived from aerial parts of bioactive methanol extract of *A. pungens* using EPM. (A) Number of entries in open arms ± S.D. and (B) Time spent in open arms (sec) ± S.D.

Using EPM, the test samples of *A. pungens* aerial pieces were assessed for their antianxiety properties. The EPM model was chosen due to its effectiveness, simplicity, ease of use, low time commitment, and lack of need for mice to have any prior training (Madaan & Sharma, 2011). The main observational basis for this paradigm is that animals exposed to approach-avoidance conflict exhibit an exploratory-cum-fear drive. When animals are on the EPM, their acrophobia—a fear of heights—causes distress. The ultimate sign of anxiety in animals is a reduction in motor activity, as indicated by the quantity of times the animal enters and stays in the open arms of EPM on average (Belzung & Griebel, 2001).

The presence of flavonoids, phenols, and triterpenoids in the bioactive extract and/or fraction of aerial parts of *A. pungens* was revealed by preliminary phytochemical tests. Our findings are consistent with the literature that has been reported to show anxiolytic activity for flavonoids such as quercetin (Saaby et al., 2009), kaempferol (Grundmann et al., 2009), chrysin (Wolfman et al., 1994), luteolin (Coleta et al., 2006), wogonin (Hui et al., 2002), bacicalin (Liao et al., 2003), apigenin (Kumar & Sharma, 2006) and triterpenoids galphimine A, galphimine B (Herrera-Ruiz et al., 2006), α , β -amyryn (Aragao et al., 2006). It is proposed that the anxiolytic activity of aerial parts of *A. pungens* is caused by flavonoids, phenols, and/or triterpenoids.

Avallone et al. (2002), Bergendorff et al. 1997), Yu et al. (2007), the serotonergic system, the regulation of

5-hydroxytryptamine1A (5HT1A), the gamma-amino butyric acid type A (GABAA) receptor system, the involvement of benzodiazepine receptors, the inhibition of γ -amino butyric acid transmission, Une et al. (2001), their capacity to raise ascorbic acid levels in the brain (Yanpallewar et al., 2005), or monoamine oxidase inhibition are some of the proposed modes of action of *A. pungens* aerial pieces' antianxiety activity.

CONCLUSION

It is finally suggested that phenolic and flavonoidal compounds were responsible for the antianxiety activity of *A. pungens* aerial parts. It is further submitted that these compounds will be isolated from bioactive fraction of plant using column chromatography studies and sophisticated flash chromatography technology in future research work.

DECLARATION OF INTEREST

The authors report no declaration of interest.

REFERENCES

1. Adwas, A.A., Jbireal, J.M. and Azab, A.E. (2019). Anxiety: Insights into Signs, Symptoms, Etiology, Pathophysiology, and Treatment. *J. Med. Sci*; Vol-2 (10), pp580-591.
2. Alekhya V, Ganapaty S, Sunitha K., 2021. Phytochemical and Pharmacological Evaluation of *Alternanthera pungens*. *Research Journal of Pharmacy and Technology*, 14, pp.7.
3. Aragao GF, Carneiro LM, Junior AP, Vieira LC, Bandeira PN, Lemos TL, Viana GS. (2006). A possible mechanism for anxiolytic and antidepressant effects of alpha- and beta-amyryn from *Protium heptaphyllum* (Aubl.) March. *Pharmacol Biochem Behav*, 85, 827-34.
4. Avallone R, Cosenza F, Farina F, Baraldi C, Baraldi M. (2002). Extraction and purification from *Ceratonia siliqua* of compounds acting on central and peripheral benzodiazepine receptors. *Fitoterapia*, 73, 390-6.
5. Belzung C, Griebel G. (2001). Measuring normal and pathological anxiety like behaviour in mice: A review. *Behav Brain Res*, 125, 141-9.
6. Bergendorff O, Dekermendjian K, Nielsen M, Shan R, Witt R, Ai J, Sterner O. (1997). Furanocoumarins with affinity to brain

Evaluation of Antianxiety profile of *Alternanthera pungens* Aerial Parts

- benzodiazepine receptors *in vitro*. *Phytochemistry*, 44, 1121-4.
- Burkill., The useful plants of west tropical Africa, Vol 3. Royal Botanic Gardens, Kew, UK 1985.
 - Coleta M, Batista MT, Campos MG, Carvalho R, Cotrim MD, Lima TC, Cunha AP. (2006). Neuropharmacological evaluation of the putative anxiolytic effects of *Passiflora edulis* Sims, its sub-fractions and flavonoid constituents. *Phytother Res*, 20, 1067-73.
 - Farnsworth NR. (1966). Biological and phytochemical screening of plants. *J Pharm Sci*, 55, 225-76.
 - Greenberg, P. E., Sisitsky, T., Kessler, R. C., Finkelstein, S. N., Berndt, E. R., Davidson, J. R. And Fyer, A. J. (1999). The economic burden of anxiety disorders in the 1990s. The Journal of clinical psychiatry.
 - Grundmann O, Nakajima J, Kamata K, Seo S, Butterweck V. (2009). Kaempferol from the leaves of *Apocynum venetum* possesses anxiolytic activities in the elevated plus maze test in mice. *Phytomedicine*, 16, 295-302.
 - Herrera-Ruiz M, Gonzalez-Cortazar M, Jimenez-Ferrer E, Zamilpa A, Alvarez L, Ramirez G, Tortoriello J. (2006). Anxiolytic effect of natural galphimines from *Galphimia glauca* and their chemical derivatives. *J Nat Prod*, 69, 59-61.
 - Hui KM, Huen MS, Wang HY, Zheng H, Sigel E, Baur R, Ren H, Li ZW, Wong JT, Xue H. (2002). Anxiolytic effect of wogonin, a benzodiazepine receptor ligand isolated from *Scutellaria baicalensis* Georgi. *Biochem Pharmacol*, 64, 1415-24.
 - Kaplan, H.I. and Sadock, B.J. (1995). Comprehensive Textbook of Psychiatry/VI. 6th ed. Williams & Wilkins, Baltimore, Maryland. pp. 1244-48.
 - Kulkarni SK. (2003). *Handbook of Experimental Pharmacology*. 3rd ed. New Delhi, India: Vallabh Prakashan, Pitampura, 135-40.
 - Kumar, S. and Sharma, A., 2006. Apigenin: The Anxiolytic Constituent of *Turneraaphrodisiaca*. *Pharmaceutical biology*, 44(2), pp.84-90.
 - Liao JF, Hung WY, Chen CF. (2003). Anxiolytic-like effects of baicalein and baicalin in the Vogel conflict test in mice. *Eur J Pharmacol*, 19, 141-6.
 - Madaan R, Sharma A. (2011). Evaluation of antianxiety activity of *Actaea spicata* Linn. *Int J Pharm Sci Drug Res*, 3, 45-7.
 - Munir, S., Gondal, A.Z., and Takov, V., (2019). Generalized anxiety disorder. <https://www.ncbi.nlm.nih.gov/books/NBK441870>.
 - Naidu, V.S.G.R., Hand Book on Weed Identification. Directorate of Weed Science Research, Jabalpur, India 2012.
 - Saaby L, Rasmussen HB, Jager AK. (2009). MAO-A inhibitory activity of quercetin from *Calluna vulgaris* (L.) Hull. *J Ethnopharmacol*, 121, 178-81.
 - Scheffer WC. (1980). *Statistics for the Biological Sciences*. Philippines: Addison-Wesley Publishing Company, 121-41.
 - Tripathi, K.D. Essentials of Medical Pharmacology. (1999). Anti Psychotic Drugs. 4th Edition. Jaypee Brothers, Medical Publishers Ltd, New Delhi. pp 414-417.
 - Une HD, Sarveiya VP, Pal SC, Kasture VS, Kasture SB. (2001). Nootropic and anxiolytic activity of saponins of *Albizzia lebeck* leaves. *Pharmacol Biochem Behav*, 69, 439-44.
 - Wild, H., Common Rhodesians weeds. Salisbury: Government Printer, 1995.
 - Wolfman C, Viola H, Paladini A, Dajas F, Medina JH. (1994). Possible anxiolytic effects of chrysin, a central benzodiazepine receptor ligand isolated from *Passiflora coerulea*. *Pharmacol Biochem Behav*, 47, 1-4.
 - Yanpallewar S, Rai S, Kumar M, Chauhan S, Acharya SB. (2005). Neuroprotective effect of *Azadirachta indica* on cerebral post- ischemic reperfusion and hypoperfusion in rats. *Life Sci*, 76, 1325-38.
 - Yu HS, Lee SY, Jang CG. (2007). Involvement of 5-HT_{1A} and GABA_A receptors in the anxiolytic-like effects of *Cinnamomum cassia* in mice. *Pharmacol Biochem Behav*, 87, 164-70.