WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

In vivo investigations of antianxiety potential of *Polypodium vulgare* L. roots in rats

Pardeep Kaur[1], Sandeep Kaur[2], Monika Bansal[3]

Akal College of Pharmacy & Technical Education, Mastuana Sahib, Sangrur, India – 148 001
Corresponding author:
Mrs. Monika Bansal; Associate Professor
E mail: monikabansal8@gmail.com

ABSTRACT :

P. vulgare roots were extracted exhaustively and successively using solvents in order of increasing polarity to prepare petroleum ether, chloroform, methanol and water extracts. It is clearly evident from preliminary phytochemical screening of petroleum ether, chloroform and water extracts did not show any presence of any main class of phytoconstituents. Because it includes flavonoids and phenolic chemicals, only methanol extract was used to screen for in vivo antianxiety efficacy utilizing the raised plus maze model. Rats were given dosages of 100, 200, or 400 mg/kg of EPM to test the antianxiety properties of a methanol extract of plant roots. Just 400 mg/kg dosages of methanol extract, out of all the tested doses, statistically equaled the conventional drug's anxiolytic efficacy while significantly increasing the number of entry and time spent in open arms in rats compared to the control. Additionally, the bioactive methanol extract was separated into a number of different fractions, including the remaining bioactive extract, n-hexane, ethyl acetate, and 1-butanol fractions. It is clearly evident from preliminary phytochemical screening of n-hexnae fraction, 1-butanol fraction and remaining bioactive extract did not show any presence of any main class of phytoconstituents. Thus, using the elevated plus maze model, only the ethyl acetate portion of the bioactive methanol extract was tested for in vivo antianxiety efficacy at dosages of 25, 50, or 100 mg/kg. Rats given 100 mg/kg doses of the ethyl acetate fraction of methanol extract showed statistically equivalent anxiolytic activity to the standard drug, but they also significantly increased the number of entries and time spent in open arms compared to the control group. Flavonoids and phenolics were found in the bioactive extract and/or fraction of plant roots, according to preliminary phytochemical investigations. Therefore, it can be concluded that these flavonoids and phenolic chemicals may be the primary components that give plant roots their antianxiety properties. Additionally, column chromatography experiments will be used to separate the kinds of chemicals that give plant roots their antianxiety properties.

Keywords: *Polypodium vulgare*, antianxiety, diazepam, elevated plus maze, flavonoids, phenols.

Introduction

P. vulgare rhizome is used in European, American, Chinese, Unani and Ayurvedic system of medicine. It is found to be efficacious in jaundice, dropsy, scurvy, and combined with mallows to remove hardness of spleen. Traditionally, it is used as nervine tonic in mental disorders. Aqueous extract of leaves and roots is found to be efficacious in malarial fever. Fresh or dried roots mix with honey is applied to nose to treat polypus. The rhizome extract was found to have antiepileptic activity (Khory and Katrak, 1981), antibiotic activity and insecticidal activities (Krishnakumaran and Schneiderman, 1968).

Keeping in view the traditional, alternative and complimentary medicinal uses, sporadic phytochemical and pharmacological reports *P. vulgare* seems to hold great potential for in depth investigation for antianxiety activity. Therefore, it was considered worthwhile to undertake biological studies, and implement the following plan of work to achieve the goal.

Materials and methods

Collection and identification of plant material

P. vulgare roots were procured from Himayala Herbs Store, Madhav Nagar, Saharanpur (Uttar Pradesh), India in November, 2024. Online literature pertaining to the microscopic characteristics of the plant was used to validate its identify.

Phytochemical studies

In a grinder, the plant roots were ground into a powder. 250 g of dried plant material was put in a thimble composed of fine filter paper. The

plant was then thoroughly extracted using petroleum ether in a Soxhlet device until a small number of droplets collected from the siphoning tube on the watch glass left no residue after evaporating. In order to obtain chloroform extract, the marc was dried, packed in a thimble, and extracted thoroughly using a Soxhlet device. Once the chloroform extraction was finished, the same process was used to obtain the methanol extract. The plant material's marc was boiled for two hours on a hot skillet with distilled water to create the water extract. A rotary vacuum evaporator was employed to recover the solvents from the crude extracts at low pressure, and a vacuum desiccator filled with fused calcium chloride was utilized to preserve the dried extracts.

A round-bottom flask containing 10 g of methanol extract was filled with 50 ml of distilled water. The suspension of extract in distilled water was made by triturating the material for 30 min with a glass rod. After that, it was heated to 50ºC for 30 minutes while being constantly stirred, and 50 ml of n-hexane was added to partition it. After cooling, the top layer, or n-hexane layer, was separated. 50 milliliters of brand-new n-hexane were added to the extract. This n-hexane partitioning process was repeated until a few milliliters of the n-hexane layer evaporated without leaving any discernible residue on the watch glass. To get the n-hexane fraction, all of the isolated n-hexane layers were combined and concentrated under lower pressure. A similar process was used to get the 1-butanol and ethyl acetate fractions from the residual bioactive extract. After successive partitioning with *n*-hexane, ethyl acetate and 1-butanol, the remaining bioactive extract was also concentrated. All the extracts of plant and fractions of the bioactive extract were screened for the presence of various groups of phytoconstituents (Farnsworth, 1966).

Antianxiety activity of various extracts of *P. vulgare* roots

Animals

Male Wistar rats weighing 250–300 g were acquired for the current studies from Akal College of Pharmacy and Technical Education in Mastuana Sahib, Sangrur. The animals were fed a typical laboratory pellet diet and had unrestricted access to water. The Institutional Animal Ethics Committee of the Akal College of Pharmacy and Technical Education, Mastuana Sahib, Sangrur, granted approval prior to the use of animals in experiments (ATRC/43/25, dated 04/06/2025). Seven days prior to the start of the trial, the animals were continuously acclimated to laboratory settings for one hour each day. According to the Committee for the Purpose of Control and Supervision on Experiments on Animals' standards, all of the experiments were conducted between 9 AM and 12 PM. In every series of studies, groups of five animals were used.

Vehicle and standard drug

The vehicle for creating the suspension of different test doses of phytomolecule-rich extracts or fractions of P. vulgare roots was distilled water with 5% Tween 80. Diazepam was administered as a typical anxiolytic medication (2 mg/kg, p.o.).

Preparation of doses

Test doses of phytomolecule-rich P. vulgare root extract (100, 200, or 400 mg/kg) and phytomolecule-rich bioactive extract fraction (25, 50, or 100 mg/kg) were made by suspending them in the vehicle at concentrations that could be given orally to rats.

Elevated plus maze model of anxiety

The plus-maze device, which was elevated (48 cm) from the floor and had two open arms (50×10 cm) and two closed arms (50×10×40 cm) with an open roof, was used to examine anxiolytic behavior in animals. Every mouse was positioned with its head facing the open arms in the middle of the elevated plus maze. The mouse's behavior during the 5-minute experiment was noted as (a) how many times it entered the open arms and (b) how long it spent there on average (average time = total time spent in open arms/number of entries in open arms). A tuberculin syringe with an oral canula was used to give the test chemicals orally. The timetable for administering the dose was so modified that, forty-five minutes after the dose was administered, each mouse took turns using the EPM device. The animals were free to interact with one another throughout the entire experiment. Every care was made to guarantee that the animals wouldn't become anxious due to anything other than the height of the plus-maze (Kulkarni, 2003).

Experimental design for the assessment of antianxiety activity

Two protocols for the experiments were created. There were ten animal groupings created, with ten animals in each group.The purpose of experimental protocol I, which included five groups, was to evaluate the antianxiety properties of P. vulgare root methanol extract.

The vehicle (0.25 ml, p.o.) was given to Group 1, the control group.

Group 2: The standard group was given diazepam intraperitoneally (2 mg/kg).

Test groups three, four, and five were given dosages of methanol extract of 100, 200, and 400 mg/kg, respectively.

The purpose of experimental procedure II, which included five groups, was to evaluate the antianxiety properties of the ethyl acetate fraction derived from the bioactive extract of P. vulgare roots.

The vehicle (0.25 ml, p.o.) was given to Group 1, the control group.

Group 2: The standard group was given diazepam intraperitoneally (2 mg/kg).

Test groups three, four, and five were given dosages of ethyl acetate fraction of 25, 50, and 100 mg/kg, respectively.

Statistics

The findings are shown as mean ⁱ standard deviation (S.D.). One-way analysis of variance (ANOVA) was used to compare the test doses with the standard and control, and then Student Newmann Keul's test was used (Scheffer, 1980).

Results and discussion

Percentage yields of various extracts of *P. vulgare* roots

The roots of P. vulgare were thoroughly and methodically extracted using solvents arranged in increasing polarity. Table 1 shows the percentage yields (% w/w) of many plant root extracts, including petroleum ether, chloroform, methanol, and water. The extraction process was complete with the help of Soxhlet technology. Soxhlet apparatus was chosen for extracting plant material because it has many advantages such as: (a) the sample is repeatedly brought into contact with the fresh portions of the solvent, thereby helping to displace the transfer equilibrium (Luque-De-Castro & Garcia-Ayus, 1998); (b) the temperature of the system remains relatively high since the heat applied to the distillation flask reaches the extraction cavity to some extent; (c) no filtration is required after leaching step; (d) sample throughput can be increased by simultaneous extraction in parallel, since the basic equipment is inexpensive; (e) very simple methodology, which needs little specialized training, has the possibility to extract more sample mass than most of the latest methods (microwave extraction and supercritical fluids).

Table 1: Percentage yields of various extracts of plant roots.

Extract	Percentage yield (%w/w)
Petroleum ether extract	4.30
Chloroform extract	2.01
Methanol extract	10.90
Water extract	16.85

Phytochemical screening of various extracts of *P. vulgare* roots

Standard specified chemical reagents were used to screen all plant root crude extracts for the presence of several types of phytoconstituents. Table 2 displays the outcomes of numerous extracts' chemical tests.

Table 2: Phytochemical screening of various extracts of plant roots.

Class of phytoconstituents	Petroleum ether extract	Chloroform extract	Methanol extract	Water extract
Carbohydrates	-	-	+	+
Proteins	-	-	-	+
Fixed oils	+	-	-	-
Alkaloids	-	-	-	-
Anthraquinone glycosides	-	-	-	-
Cyanogenetic glycosides	-	-	-	-
Cardiac glycosides	-	-	-	-
Flavonoids	-	-	+	-
Saponins	-	-	+	+
Tannins	-	-	+	-
Steroids/Triterpenoids	-/-	+/-	-/-	-/-
Coumarins	-	-	-	-

+ Present, - Absent

In vivo antianxiety activity of methanol extract of *P. vulgare* roots

Although water has extracted more constituents as evident from percentage yield of water extract, but methanol extract showed presence of major classes of phytoconstituents. Therefore methanol extract was further subjected to antianxiety activity using well established model named elevated plus maze model.

Furthermore, table 6 makes it abundantly clear that no major class of phytoconstituents was detected in the petroleum ether, chloroform, and water extracts after preliminary phytochemical screening. Therefore, using the raised plus maze model, only methanol extract was tested for in vivo

antianxiety efficacy.

Rats were given EPM to test the methanol extract of plant roots for antianxiety properties. Following the administration of 100, 200, or 400 mg/kg doses of crude methanol extract, diazepam (2 mg/kg), and vehicle, Table 3 displays the average number of entries and mean duration of time spent in the open arms of EPM by rats. When compared to the control, 400 mg/kg of methanol extract had the most antianxiety effects among the different test doses. It was shown that additional dosages of 100 or 200 mg/kg showed only modest antianxiety effects. When compared to the control group, rats given 100 or 200 mg/kg demonstrated a markedly higher number of entries and time spent in the raised plus maze model's open arms; nonetheless, their activity level was not on par with that of the standard group. Although animals given 400 mg/kg dosages of methanol extract had much more entrances and spent more time in open arms than rats given the control, only at this dose did the rats exhibit statistically equal anxiolytic activity to the conventional medication. Finally, it may be concluded that the methanol extract exhibited dose-dependent antianxiety effect.

Table 3: Methanol extract of plant roots' antianxiety properties utilizing the raised plus maze model.

Treatment	Dose (mg/kg)	Number of entries in open arms ± S.D.	Time spent in open arms (sec) ± S.D.
Control	Vehicle	3.20 ± 0.83^{a}	5.20 ± 0.83^{a}
Diazepam	2	$9.80 \pm 1.09^{*}$	$16.00 \pm 0.70^{*}$
Methanol extract	100	$4.80 \pm 0.83^{*a}$	$7.80 \pm 0.83^{*a}$
	200	$7.00 \pm 0.70^{*a}$	$10.40 \pm 1.14^{*a}$
	400	$\mathbf{9.60 \pm 0.54^{*}}$	$\mathbf{15.40 \pm 1.14^{*}}$

*P<0.05 versus Control; aP<0.05 vs Diazepam (Standard medication); one-way ANOVA followed by Student-Newman-Keul's test; n=10; Mean ± S.D.

Percentage yields of various fractions of bioactive methanol extract of plant roots

Table 4 displays the percentage yields (% w/w) of the different fractions—n-hexane, ethyl acetate, 1-butanol, and the remaining bioactive extract—obtained from the bioactive methanol extract.

Table 4: The percentage yields of the different fractions derived from the bioactive methanol extract of plant roots.

Fraction	Percentage yield* (% w/w)
n-Hexane fraction	2.15
Ethyl acetate fraction	10.58
1-Butanol fraction	20.89
Remaining bioactive extract	61.25

*Calculated via methanol extract yield

Phytochemical screening of various fractions of bioactive methanol extract of plant roots

Table 5 displays the findings of a preliminary phytochemical screening of several fractions made from a bioactive methanol extract of plant roots.

Table 5: Different fractions from the bioactive methanol extract of plant roots were screened phytochemically.

Class of phytoconstituents	*n*-Hexane fraction	Ethyl acetate fraction	1-Butanol fraction	Remaining bioactive extract
Flavonoids	-	+	-	-
Tannins	-	+	-	-
Saponins	-	-	+	+
Carbohydrates	-	-	+	+

+ Present, - Absent

In vivo antianxiety activity of ethyl acetate fraction of bioactive methanol extract of plant roots

It is clearly evident from table 5 that preliminary phytochemical screening of n-hexnae fraction, 1-butanol fraction and remaining bioactive extract did not show any presence of any main class of phytoconstituents. Therefore, only ethyl acetate fraction of bioactive methanol extract was subjected to screen in vivo antianxiety activity using elevated plus maze model.

Rats were given EPM to test for antianxiety properties in the ethyl acetate fraction of the methanol extract of plant roots. Following the administration of 25, 50, or 100 mg/kg dosages of the ethyl acetate fraction of methanol extract, diazepam (2 mg/kg), and vehicle, Table 6 displays the average number of entrances and mean duration of time spent in the open arms of EPM by rats. When compared to the control, 100 mg/kg of the ethyl acetate fraction of methanol extract had the most antianxiety effects among the different test doses. It was discovered that additional dosages of 25 or 50 mg/kg showed only modest antianxiety effects. Although the activity was not comparable to that of the standard group, rats treated with 25 or 50 mg/kg shown a substantial increase in the number of entries and time spent in the elevated plus maze model's open arms when compared to the control group. Although rats' frequency of entry and time in open arms was dramatically increased by 100 mg/kg dosages of the ethyl acetate fraction of methanol extract compared to the control, only at this dose was statistically equivalent anxiolytic activity to the standard medication seen. Finally, it may be concluded that the methanol extract's ethyl acetate fraction exhibited dose-dependent antianxiety efficacy.

Table 6: Antianxiety activity of ethyl acetate fraction of methanol extract of plant roots using elevated plus maze model.

Treatment	Dose (mg/kg)	Number of entries in open arms ± S.D.	Time spent in open arms (sec) ± S.D.
Control	Vehicle	3.40 ± 0.54^{a}	5.00 ± 0.70^{a}
Diazepam	2	$8.60 \pm 1.14^{*}$	$15.20 \pm 0.83^{*}$
Ethyl acetate fraction	25	$4.00 \pm 0.70^{*a}$	$7.20 \pm 0.83^{*a}$
	50	$5.80 \pm 0.83^{*a}$	$11.20 \pm 0.83^{*a}$
	100	**$8.20 \pm 0.83^{*}$**	**$14.60 \pm 0.54^{*}$**

n=10; 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.

Conclusion

Because it is efficient, straightforward, time-efficient, manageable, and doesn't require any prior training for the rats, the EPM model was chosen (Madaan & Sharma, 2011). The main premise of this paradigm is the fact that animals exposed to approach-avoidance conflict exhibit an exploratory-cum-fear drive. When placed on the EPM, the animals experience uneasiness owing to acrophobia, a fear of height. Reduced motor activity, as indicated by the number of entries and average amount of time spent in the open arms of EPM by the animal, is the final sign of anxiety in the animals (Belzung & Griebel, 2001).

Preliminary phytochemical studies showed presence of flavonoids and phenolic in bioactive extract and / or fraction of plant roots. Our results are in agreement with the reported literature where flavonoids and phenolic compounds – 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); maltol, gallic acid, *p*-coumaric acid, hesperitin (Kumar and Kumar, 2017) have been reported to exhibit anxiolytic activity. It is suggested that flavonoids and / or phenolic are responsible for anxiolytic activity of roots.

The suggested modes of actions for antianxiety activity of plant roots are involvement of serotonergic system (Kim et al., 2004), regulation of 5-hydroxytryptamaine1A ($5HT_{1A}$), gamma-amino butyric acid type A ($GABA_A$) receptor system (Yu et al., 2007), involvement of benzodiazepine receptors (Avallone et al., 2002; Bergendorff et al., 1997), inhibition of γ-amino butyric acid transmission (Une et al., 2001), their ability to increase ascorbic acid level in brain (Yanpallewar et al., 2005) or monoamine oxidase inhibition (Saaby et al., 2009).

Therefore, it can be concluded that these flavonoids and phenolic chemicals may be the primary components that give plant roots their antianxiety properties. Additionally, column chromatography experiments will be used to separate the kinds of chemicals that give plant roots their antianxiety properties.

REFERENCES

1. Avallone R, Cosenza F, Farina F, Baraldi C, Baraldi M. Extraction and purification from *Ceratonia siliqua* of compounds acting on central and peripheral benzodiazepine receptors, Fitoterapia, 2002; 73: 390.
2. Belzung C, Griebel G. (2001). Measuring normal and pathological anxiety like behaviour in mice: A review. *Behav Brain Res*, 125, 141-9.
3. Bergendorff O, Dekermendjian K, Nielsen M, Shan R, Witt R, Ai J, Sterner O. Furanocoumarins with affinity to brain benzodiazepine receptors *in vitro*, Phytochemistry, 1997; 44: 1121.
4. 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.
5. Farnsworth NR. (1966). Biological and phytochemical screening of plants. *J Pharm Sci*, 55, 225-76.

6. 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.

7. 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.

8. Khory, R.N; Katrak, N.N; 1981. Meteria medica of India and their therapeutics. Neeraj Publication House, New Delhi.

9. Kim WK, Jung JW, Ahn NY, Oh HR, Lee BK, Oh JK, Cheong JH, Chun HS, Ryu JH. Anxiolytic-like effects of extracts from *Albizzia julibrissin* bark in the elevated plus-maze in rats, Life Sci, 2004; 75: 2787.

10. Krishnakumaran, A; Schneiderman, H; 1968. Chemical control of moulting in arthropods. Nature. 220. 601-603p.

11. Kulkarni SK. (2003). *Handbook of Experimental Pharmacology*. 3rd ed. New Delhi, India: Vallabh Prakashan, Pitampura, 135-40.

12. **Kumar D,** Kumar S. Isolation and characterization of bioactive phenolic compounds from *Abies pindrow* aerial parts. Pharmaceutical Chemistry Journal, 2017, 51(3), 205-210.

13. Kumar S, Sharma A. (2006). Apigenin: The anxiolytic constituent of *Turnera aphrodisiaca*. *Pharm Biol*, 44, 84-90.

14. 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.

15. Luque-de-Castro MD, Garcia-Ayus LE. (1998). Soxhlet extraction of solid materials: An out dated technique with a promising innovative future. *Anal Chem Acta*, 369, 1-10.

16. Madaan R, Sharma A. (2011). Evaluation of antianxiety activity of *Actaea spicata* Linn. *Int J Pharm Sci Drug Res*, 3, 45-7.

17. Saaby L, Rasmussen HB, Jager AK. (2009). MAO-A inhibitory activity of quercetin from *Calluna vulgaris* (L.) Hull. *J Ethnopharmacol*, 121, 178-81.

18. Scheffer WC. (1980). *Statistics for the Biological Sciences*. Philippines: Addison-Wesley Publishing Company, 121-41.

19. 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.

20. 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.

21. 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.