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ORIGINAL ARTICLE

Central Nervous System Activity of Phenol-Rich Fraction of *Piper sylvaticum* Roots

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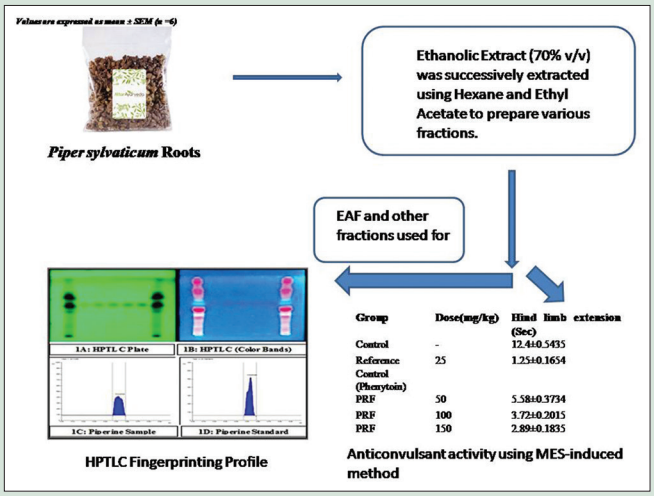
ABSTRACT

Obective: Piper sylvaticum Roxb. is an important folk medicine in Indian Traditional System of Medicine widely used by different tribes in many countries. In the present study, the anticonvulsant activity of extract/fractions of *Piper sylvaticum* (PS) roots was investigated. **Materials and Methods:** 70% ethanolic extract of PS roots was successively extracted using hexane and ethyl acetate to prepare various fractions. Total phenol content was found at maximum 324.65 mg/gallic acid equivalent/g in ethyl acetate fraction (EAF) (phenol-rich fraction [PRF]). High-performance thin-layer chromatography fingerprinting profiling of PS roots was performed. The anticonvulsant properties of the EAF of roots of PS were examined by maximal electroshock method as compared to standard phenytoin (25 mg/kg body weight). **Result:** It was found that EAF shows potent anticonvulsant activity at different dose levels against maximum electroshock seizure-induced convulsions in Swiss albino mice. **Conclusion:** From the observation, it can be concluded that the current study has expressed that the phenol-rich EAF of the ethanolic extract of the roots of PS has shown the dose-dependent anticonvulsant effect in mice. The anticonvulsant potential may be due to the presence of phenolic compounds in PRF. The outcomes suggested a high potential for application of EAF of PS root as an anticonvulsant agent.

Key words: Anticonvulsant activity, high-performance thin-layer chromatography, *Piper sylvaticum,* piperine

SUMMARY

- Phenol-rich fraction and other fractions/extract of *Piper sylvaticum* (PS) roots were screened for anticonvulsant activity as well as high-performance thin-layer chromatography fingerprinting profile of PS roots was studied
- Significant anticonvulsant activity was found while treating with ethyl acetate fraction.



Abbreviations Used: HPTLC: High-performance thin-layer chromatography, OECD: Organization for Economic Co-operation and Development, GAE: Gallic acid equivalent, PRF: Phenol-rich fraction.

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INTRODUCTION

Natural products from plants are important sources of new drugs.[1] The members of genus Piper L. include nearly 2000 species scattered in pantropical areas which are a potential source of remedies in traditional medicine.[2] In India, this genus is signified by more than 100 species mainly scattered in the Western Ghats and Eastern Himalayan region, with about 65 species occurring in northeastern states. The *Piper* species have high commercial, economic, and medicinal importance. Economically, the Piperaceae is significant for the pepper in the global spice markets. Plants belonging to the genus Piper are believed in the Indian Ayurveda system of medicine for their therapeutic properties and traditional medicine of Latin America and West Indies.[3,4] *Piper sylvaticum* Roxb., belonging to family Piperaceae, is known by other names, namely Pahari pipul (Bengali, Assamese) and Mountain long pepper (English). *P. sylvaticum* (PS) is known to contain compounds such as piper amides: piperine, lignans such as cubebin and sesamin, flavones and chalcones such as 4', 7-dimethoxy-5-hydroxyflavone, flavokawain, sylvatin, pipataline, tectochrysin, 5-hydroxy-7, 3', 4'-trimethoxyflavone, piperine, piperlonguminine, n-isobutyldeca-trans-4 dienamide, and β-sitosterol.[5-7] Seeds of PS contain lignans, sylvatin-4'-7-dimethoxy-5-hydroxy flavone, and (+)-Sylvone.[8,9] Chemical and spectroscopic study has fully characterized two constituents of the seeds of PS which include the new epieudesmin type lignan sylvatesmine and 3, 5-dihydroxy-4, 7-dimethoxy flavones.[10] PS fruits are used as carminative and antidote to snake poison.[11] Roots of the aforementioned plant are very useful in the examination of enlargement of spleen and liver and all types of complications related to digestive tract.[12] PS is also known to possess activity for the treatment of chronic kidney diseases, anticancer activity, as well as central nervous system stimulant activity, especially on the respiratory center.[8,13,14]

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Seizure refers to an occasioned excessive discharge at nerve tissue. This is distinguished by a spontaneous retrenchment of the muscles producing scowl of the body and limbs. Several traditional herbs are remarkably valuable and vital in the pursuit of seizure management and probably future drug development. In animal models, the efficacy of herb in the management of convulsion is determined by its capability to interrupt the inception of seizure, delay fatality, or shield the animals. The delay in the start of the clonic or tonic seizure is due to the ability of plant extract to elevate seizure threshold.[15] Epilepsy is the commonest neurological state, differentiated by spontaneous periodic seizures, generated by irregular electrical activity in the brain cortex. The contribution of hyperexcitable neurons links the pathogenesis of epilepsy and the production of coordinated neuronal activity with an unevenness between inhibitory gamma aminobutyric acid-mediated and excitatory (glutamate mediated) neurotransmission.[16] However, there is no single report yet demonstrated on PS for anticonvulsant potential against maximal electroshock (MES)-induced seizure response. On the other hand, only a few and very earlier studies are related to piperine anticonvulsant effects and its possible mechanism of action.[17] Therefore, the aim of this study was to evaluate anticonvulsant activity of the different fractions of roots of PS using MES-induced seizure in Swiss albino mice as well as to study the high-performance thin-layer chromatography (HPTLC) fingerprinting profile of roots of PS.

MATERIALS AND METHODS

Collection and authentication of plant material

PS roots were collected from Kolkata, West Bengal, India, and authenticated by Taxonomic division of National Botanical Research Institute, Lucknow. and a voucher specimen was deposited for future reference.

Extraction and preparation of phenolic-rich fraction

PS root powder (100 g) was macerated in 70% ethyl alcohol (1:5 w/v) at 25°C. The supernatants were decanted after, 24 h, and the sediments were re-soaked in the corresponding fresh solvent. The procedure was repeated three times for complete extraction. Supernatants were then collected separately, followed by filtration and centrifugation at 5000 rpm for 10 min at 4°C. Extract was then lyophilized and the dried extracts were preserved in hermetically sealed dark bottles at 4°C. 5 g of raw extract was dissolved in 100 ml water and successively extracted three times applying 100 ml hexane to get hexane fraction (HF) and later using 100 ml ethyl acetate to get ethyl acetate fraction (EAF).

Determination of total phenol content

In brief, 150 µl of extract/fractions of PS roots and 2400 µl of three times distilled water plus 150 µl of 0.25 N Folin–Ciocalteu's reagent were mixed well. The fusion was allowed to react for 3 min, and later 300 µl of 1 N sodium carbonate solution was added and stirred. The mixture obtained in the form of the solution was incubated at 25°C in the dark for 2 h. The absorbance was calculated at 725 nm using a double-beam spectrophotometer, and the outcomes were revealed in mg of gallic acid equivalents (GAEs) per gram of extract/fraction.[18]

Characterization of piperine by high-performance thin-layer chromatography fingerprinting

Preparation of standard solutions

Stock solutions lupeol and ursolic acid were prepared separately by dissolving those 0.1 mg/mL in methanol.

Sample preparation

The roots of PS were collected and thoroughly washed with water to remove all debris. The plant material was shade dried and powdered by using the electric grinder at 60 mesh size. Extraction was performed by soxhlation method. First, the powdered plant material was defatted under soxhlet assembly using 250 mL of 98% petroleum ether for 6 h. Defatted powdered drug was successively extracted by Soxhlet extractor by using 250 mL chloroform, followed by methanol up to 9 h. The final methanolic fraction obtained was passed through Whatman filter paper. The filtrate obtained was concentrated under vacuum in a rotary evaporator at 40°C and stored at 4°C for further use. The dried extracts were dissolved in 98% methanol to obtain a stock solution of 10 mg/mL, which is used for application of spots on HPTLC plates.

Instrumentation and chromatographic conditions

The following were the instruments and chromatographic conditions used: spotting device: Linomat V automatic sample applicator; CAMAG (Muttenz, Switzerland), syringe: 100 µL Hamilton (Bonaduz, Switzerland). TLC chamber: glass twin trough assembly (20 cm × 10 cm × 4 cm); CAMAG. Densitometer: TLC Scanner 3 linked to winCATS software V.4.06 (CAMAG Scientific Inc., Wilmington, N.C., USA); CAMAG. HPTLC plates: 20 cm × 10 cm, 0.2 mm thickness precoated with silica gel 60 F254; E. Merck (Darmstadt, Germany). Experimental conditions were as follows: temperature, 25°C ± 2°C; relative humidity, 40%. Solvent system: toluene–ethyl acetate–formic acid (7:3:0.1). Detection wavelength: 550 nm for lupeol and 522 nm for ursolic acid. Slit dimension: 5.00 mm × 0.45 mm. Scanning speed: 10 mm s^{-1} and source of radiation: Deuterium lamp.

Biological activity

Animals

Swiss albino mice estimating 18–25 g of either sex were used for the study. They were separately housed. Animals were administered with standard rodent pellet diet, and the diet was withdrawn 18–24 h before the experiment, though the water was allowed *ad libitum*. All studies were performed under the guidelines for the care and handling of laboratory animals, as chosen and declared by the Institutional Animal Care Committee, CPCSEA, India (Reg. No. 222/2000/CPCSEA). All the chemicals employed were of analytical grade from standard companies, and the water represents double-distilled water. A standard orogastric cannula was used for oral drug administration.

Acute toxicity study

The acute toxicity of EAF of PS roots was arranged by utilizing Wistar albino rats (180–220 g), kept under standard husbandry conditions. Acute toxicity was calculated as per the Organization for Economic Co-operation and Development guidelines 420 (fixed-dose method). After administration of dose 2, g/kg body weight (bw) of EAF of the mortality with each treatment was noted after 14 days.

Anticonvulsant activity

Maximal electroshock convulsion model

A total of thirty Swiss albino mice were divided into six groups of six mice each. After the administration of the particular treatments, all groups were tested after 30 min for MES-induced seizure response. All the experimental groups were compared with the control treated with vehicle.

Experimental design

- Group I normal control: 2% Tween 80 solution, 0.5 ml/kg bw per oral
- Group II reference control: Phenytoin, 25 mg/kg bw injected i.p

- Group III phenol-rich fraction (PRF), 50 mg/kg bw orally as a suspension in 2% Tween 80 solution
- Group IV PRF, 100 mg/kg bw orally as a suspension in 2% Tween 80 solution
- Group V PRF, 150 mg/kg bw orally as a suspension in 2% Tween 80 solution.

Statistical analysis

The mean values ± standard deviation were determined for each parameter. For determining the significant intergroup difference, each parameter was analyzed separately, and one-way analysis of variance was performed. Then, the specific comparisons of the group mean values were made using Dunnett's test procedure. All the analyses were carried out using Graphpad Prism 7 software (Graphpad Prism Software, CA, USA).

RESULTS AND DISCUSSION

Phenol-rich fraction

We used 70% ethanol as our extraction solvent and got 20.35% yield of extracted matter from PS roots. From this crude extract phenol rich extract (PRE), PRF was prepared by sequential extraction using hexane and ethyl acetate. The total phenol content of crude extract/ethanol extract (PRE), HF, ethyl acetate fraction (EAF), and aqueous fraction was found to be 254.54, 194.84, 324.65, and 210.76 (mg/GAE/g extract/fraction), respectively. These results indicate that EAF contained the maximum amount of total phenolic compounds. Therefore, EAF is considered as PRF and it was used for the cytotoxic and anticonvulsant activities.

Quantitative estimation by high-performance thin-layer chromatography

HPTLC of plant sample is illustrated in Figure 1. While performing quantitative evaluation of piperine in the extract of PS roots using HPTLC, the piperine content was found to be 0.06% as shown in Figure 1a-d.

Acute toxicity

Over the study term of 14 days, no fatality was seen up to dose of 2 g/kg bw of the EAF of PS roots orally. During the observation time, animals did not produce any changes in the general appearance.

Anticonvulsant activity

Numerous synthetic anticonvulsant drugs are presently available in the market for applying in the management, control, and cure of patients with epilepsy. The majority of the synthetic drugs are not only difficult to get and high priced, but also have several harmful adverse effects. Thus, there is a huge requirement for the development of inexpensive, useful, and harmless anticonvulsant drugs from plants and other sources. The present study revealed that in control group, which was provided 2% Tween 80 solution, hind limb extension time was found to be 12.4 ± 0.5435 s. While in reference control group treated with phenytoin, 25 mg/kg bw i.p, hind limb extension time was found to be 1.25 ± 0.1654 s. PRF from ethanolic extract of PS roots at doses of 100 and 150 mg/kg has shown significant reduction in the duration of convulsions (3.72 ± 0.2015 and 2.89 ± 0.1835), respectively, as shown in Table 1.

CONCLUSION

The current study has expressed that the PRF of the ethanolic extract of the roots of PS has shown the dose-dependent anticonvulsant effect in mice. The anticonvulsant potential may be due to the presence of phenolic compounds in PRF. The outcomes suggested a high potential for application of EAF of PS root as an anticonvulsant agent. Currently, there is a great availability of drugs in clinics for epilepsy management. However, most of them are alternatives or 2nd choices, pointing out to the need for new safer and more efficacious drugs for those neurologic conditions, this drug may be a better choice. It can also be incorporated in nutraceuticals with notable benefits for humankind or animal health.

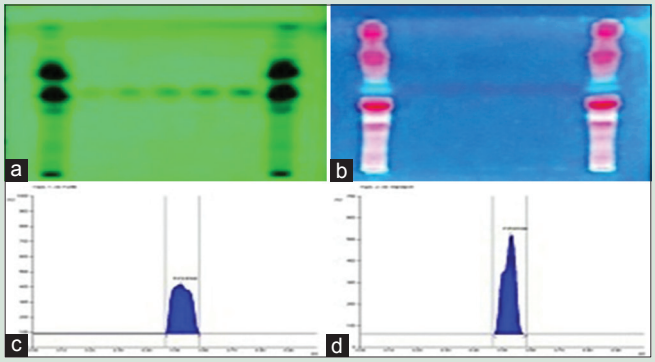

Figure 1: (a-d) Quantitative estimation of piperine in extract of *Piper sylvaticum* roots using high-performance thin-layer chromatography

Table 1: Effect of phenol-rich fraction of *Piper sylvaticum* roots on hind limb extension induced by maximal electro shock in mice

Group	Dose (mg/kg)	Hind limb extension (s)
Control	-	12.4±0.5435
Reference control (phenytoin)	25	1.25±0.1654
PRF	50	5.58±0.3734
PRF	100	3.72±0.2015
PRF	150	2.89±0.1835

Values are expressed as mean±SEM (n=6). PRF: Phenol-rich fraction; SEM: Standard error of mean

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat Prod 2012;75:311-35.
2. Quijano-Abril MA, Callejas PR, Miranda-Esquivel DR. Areas of endemism and distribution patterns for Neotropical *Piper* species (*Piperaceae*). J Biogeogr 2006;33:1266-78.
3. Kirtikar KR, Basu BD. Indian Medicinal Plants. Vol. 3. LM Basu, Allahabad, India; 1933. p. 2128.
4. CSIR. Anonymous. The Wealth of India: Raw Materials. Vol. 3. New Delhi: CSIR; 1969. p. 83.
5. Banerji A, Ghose PC. Sylvatine A new alkamide from *Piper sylvaticum*. Tetrahedron 1973;29:977-9.
6. Malhotra S, Koul SK, Taneja SC, Pushpangadan P, Dhar KL. A neolignan from *Piper sumatranum*. Phytochem 1990;29:2733-4.
7. Mahanta PK, Ghanim A, Gopinath KW. Chemical constituents of *Piper sylvaticum* (Roxb) and Piper boehmerifolium (Wall). J Pharm Sci 1974;63:1160-1.
8. Banerji J, Dhara KP. Lignan and amides from *Piper sylvaticum*. Phytochem 1974;13:2327-8.
9. Banerji A, Sarkar M, Ghosal T, Pal SC, Shoolery JN. Sylvone, a new furanoid lignan of *Piper sylvaticum*. Tetrahedron 1984;40:5047.

10. Banerji A, Pal SC. A New alkamide from *Piper sylvaticum*. Phytochem 1982;21:6:1321-3.
11. Chopara RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. 6th ed. New Delhi: National Institute of Science Communication and Information Resources; 2002. p. 194.
12. Shastri AD, Lochan KA. Bhaisajya Ratnavali of Govinda Dasji. Vol. 1. Varanasi, India: Head office Chaulkhamblta Sanskrit Sansthan; 2006. p. 608.
13. Touwaide A, De Santo NG, Aliotta G. The origins of western herbal medicines for kidney diseases. Adv Chronic Kidney Dis 2005;12:251-60.
14. Graham JG, Quinn ML, Fabricant DS, Farnsworth NR. Plants used against cancer – An extension of the work of Jonathan Hartwell. J Ethnopharmacol 2000;73:347-77.
15. Balamuruga G, Muralidharan P, Selvaraja S. Antiepileptic activity of poly herbal extract from Indian medicinal plants. J Sci Res 2009;1:153-9.
16. Dalby NO, Mody I. The process of epileptogenesis: A pathophysiological approach. Curr Opin Neurol 2001;14:187-92.
17. D'Hooge R, Pei YQ, Raes A, Lebrun P, van Bogaert PP, de Deyn PP, *et al.* Anticonvulsant activity of piperine on seizures induced by excitatory amino acid receptor agonists. Arzneimittelforschung 1996;46:557-60.
18. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic 1965;16:144-58.