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Pharmacognostic study of *Eranthemum nigrum* Root

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ABSTRACT:
Objective: *To analyze the pharmacognostic characteristics and physicochemical parameters of the root of Eranthemum nigrum (E. nigrum).*

Methods: *Microscopic characters and powder analysis had been carried out with the help of a microscope. The physicochemical properties such as loss on drying, total ash value, acid insoluble ash value, water-soluble ash value, extractive values, and fluorescence of E. nigrum had been performed.*

Results*: The color, shape, size, odor, and surface characteristics were reported from the root and powdered root material of E. nigrum. Light microscope images of cross-section and powdered root revealed lignified xylem fibers, xylem vessels, cork cells, and parenchyma cells. Phytochemical testing confirmed steroids, alkaloids, tannins, saponins, carbohydrates, glycosides, amino acids, and proteins. Physicochemical parameters such as moisture content, ash value, extractive value, and fluorescent behavior of root powder have also been established.*

Conclusion: *The morphological, microscopical, and physicochemical parameter results provided in this paper may be utilized as a basis for the preparation of a monograph on E. nigrum root.*

Keywords: *Pharmacognostic, Eranthemum nigrum, Diacytic stomata, Lignified xylem vessels, Phytochemical and Physicochemical analysis.*

INTRODUCTION

Medicinal plants play a significant part in traditional medicines intended for the therapy of various health issues. However, a crucial hurdle, which has impeded the promotion of alternative medications in the developed countries, is the lack of documentation and the absence of stringent quality control measures. Additionally, there is a dependence on all study data meted out on traditional medicines by way of documentation. Keeping this issue, it is now quite necessary to generate assurance about the standardization of the plant and its parts to be used as a medication. During standardization, we can take advantage of various techniques and methodologies to achieve our goal in a phase-wise approach, e.g., pharmacognostic and phytochemical studies.
These techniques and methods are helpful in the recognition and standardization of the plant material. Appropriate characterization and quality assurance of starting material are crucial to ensure the reproducible quality of herbal medicine to assist people in justifying its safety and effectiveness. Because of this reason, we have executed pharmacognostic studies of *Eranthemum nigrum* belongs to the family Acanthaceae (1). This sort of research is not going to help in authentication but additionally ensures the reproducibility of herbal goods in promoting (2).

In the present study, we have been focusing our exploration on one of the commonly available plants in India, i.e., *Eranthemum nigrum* belongs to the family Acanthaceae. The family Acanthaceae consists of almost 4000 species of exotic plants. Various species of Genus *Eranthemum* being utilized traditionally for extensive kinds of ethnomedicinal purposes. The genus *Eranthemum*, with around 138 species, some of the important species include *E. austrosinensis, E. burmanicum, E. capense, E. ciliatum, E. erythrochilum, E. griffithii, E. macrophyllum, E. macrostachyus, E. obovatum, E. pulchellum, E. purpurascens, E. roseum, E. strictum, E. tapingense, E. tubiflorum*, and *E. watti*. The *Eranthemum nigrum* (Acanthaceae) is native to the Pacific Islands. The shrub attains a height of 1.5-1.8 m. The upper surface of leaves is blackish purple and the lower surface purplish with dark veins. The flowers are in erect terminal spikes, white and spotted rose at the base (3). Plants are adapted to partial shade. The leaves are elliptical, glossy, or dull with smooth margins and acute tips (4, 5). All parts of this plant are widely used as a folklore medicine for the treatment of various ailments by the Indian traditional healer. Ethnomedicinally, the genus *Eranthemum* has been documented various pharmacological activities, including antipyretic (6), antidiabetic (7), antiulcer (8), antimicrobial (9), larvicidal, ovicidal, and pupicidal against *Anopheles*

stephensi (10), gastroprotective (11) and anti-inflammatory (12).

A Literature study and screening of scientific data says a lot of native medicines have already been investigated as regards their botany and chemistry is concerned. However, a systematic standardization, including Pharmacognostical and physicochemical study, is still lacking. The present investigation of *Eranthemum nigrum*.(Acanthaceae) is therefore taken up to establish certain botanical and chemical standards that would help in crude drug identification as well as in checking adulteration, if any. Further, the study will greatly help in quality assurance of finished products of herbal drugs (13, 14)

MATERIALS AND METHODS

Plant Collection and Authentication

The plant was obtained from V. V. Institute of Pharmaceutical Sciences, Gudlavalleru, Krishna District of Andhra Pradesh, India, during the month of September 2017 and authenticated by Dr. K. Madhava Chetty, Taxonomist at Sri Venkateswara University, Tirupati, India. The plant was deposited at the herbarium for future reference. One portion of the root is preserved in formalin: acetic acid: alcohol mixture for histological studies and the remaining portion was shade dried, powdered and sieved through 20 mesh and kept in an airtight container for future use.

Chemicals

All analytical grade chemicals were utilized in this study were procured from E. Merck, Germany. Absolute alcohol, phloroglucinol, acetic acid, chloral hydrate, H_2SO_4, NaOH, HNO_3, $FeCl_3$, distilled water, Conc. HCl and chloroform.

Pharmacognostic evaluation

Morphological evaluation

Organoleptic evaluation of *E. nigrum* root has been carried out in accordance with the color, size, odor, shape, and taste as per WHO Quality Control methods of herbal medicine (15).

Microscopic evaluation

Preparation of sections

Microscopic studies had been done by preparing a thin hand section of the root with the help of a sharp cutting edge of the blade, then cleared with chloral hydrate solution, stained with phloroglucinol-hydrochloric acid (1:1), and mounted in glycerin.

Powdered microscopy

The powder microscopy was carried out in accordance with the procedure described in Khandelwal (16).

Quantitative analysis

The quantitative examinations, including stomatal number, stomatal index, vein islet number, and vein termination number, were studied using the standard method (2).

Preparation of extracts and preliminary phytochemical analysis

The powdered material had been extracted with various solvents according to its polarity, i.e., chloroform, methanol, and water. 5 g root powder was extracted with 20 ml of the respective solvent by maceration at room temperature for 24 hours. Then, filtered through Whatman filter paper and collect the filtrate, concentrated with rota-evaporator. Then, the extracts had been subjected to preliminary phytochemical screening according to standard methods (16, 17).

Physicochemical analysis

Physicochemical parameters such as ash value, moisture content, and extractive values were determined according to the procedures mentioned in WHO quality control methods for herbal materials (18).

Fluorescence analysis

Various reagents were utilized to check the fluorescence activity. In this, 0.1 g of root powder was blended with 1.5 ml of respective reagent (Table 4). The mixture was placed on a slide for a minute and observed under visible light, short ultra-violet light (254 nm), and long ultraviolet light (365 nm) (19).

RESULTS

Morphological characteristics

The morphological characteristics of *E. nigrum* root were described in Figure 1 and Table 1.

Figure 1: Morphological features of Root of *Eranthemum nigrum*

Table1: Morphological Characteristics of Root of *Eranthemum nigrum*

Characters	Observation
Colour	Buff
Odor	Characteristic
Taste	Characteristic
Texture	Smooth
Thickness	0.2-4 cm

Anatomical Description

Root

The transverse section of the root of *E. nigrum* showed the presence of Cortex (Phellem) shows 3-4 polygonal thick-walled parenchymatous cells filled with brown content. Cork cambium (Phellogen) was made up of 3-5 layered narrow, tangentially elongated parenchymatous cells. The secondary cortex (Phelloderm) is 4-6 layered rows of tangentially elongated thin-walled cells. The endodermis showed the presence of Phloem and xylem. The Phloem is present in between the medullary rays. The medullary rays are parenchymatous and are uniseriate to triseriate, majorly biseriate. Radially arranged vascular bundles were present in which, Phloem is well developed and shows the presence of phloem fibers, which are non-lignified. It also showed the presence of phloem parenchyma. The xylem region was similar to the phloem region and was also surrounded by uniseriate to triseriate medullary rays. Xylem tissue consists of spiral xylem vessels, xylem fibers, and xylem parenchyma (Figure 2).

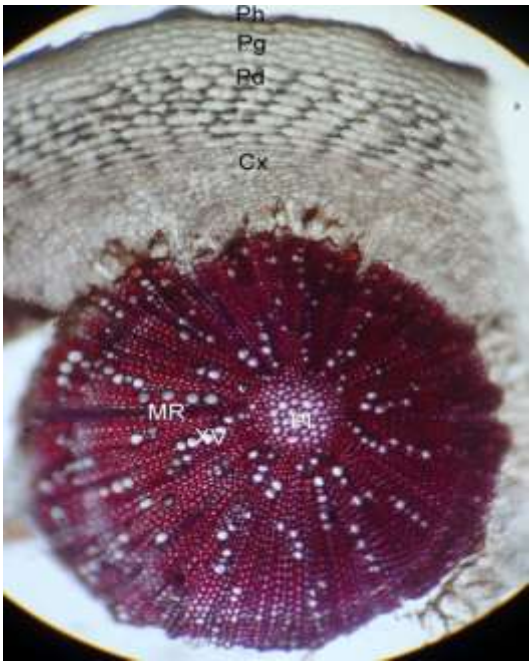

Figure 2: Transverse Section of Root of *Eranthemum nigrum;* Ph: Phellem; Pg: Phellogen; Pd: Phelloderm Cx: Cortex; MR: Medullary rays; XV: Xylem Vessels; XP: Xylem parenchyma; P.I.: Pith.

Figure 3: T.S of *Eranthemum nigrum* root showed Medullary rays and Xylem Vessels. MR: Medullary rays; XV: Xylem vessels.

Figure 4: Detailed T.S of Root showed the Central region of Eranthemum *nigrum*. MR: Medullary rays; XV: Xylem vessels; P.I.: Pith.

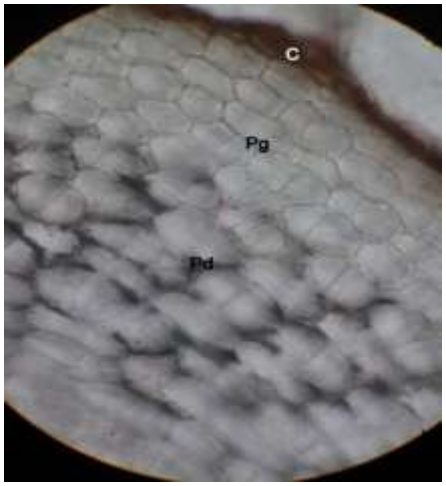

Figure 5: T.S section of *Eranthemum nigrum* root showed cork region. C: Cork; Pg: Phellogen; Pd: Phelloderm

Powder microscopy

The crude powder of root was buff in color with characteristic odor and taste. The microscopic study of the powder showed revealed different characters such as diacritic stomata, covering trichomes, xylem vessels, and parenchyma cells (Figure 8).

Figure 8: Powder Microscopy of Root of *Eranthemum nigrum* (a)Parenchyma cells (b) lignified xylem vessels (c) Cork cells (d) Medullary rays with xylem vessels

Preliminary phytochemical analysis

The results of the qualitative phytochemical analysis of crude powder of *E. nigrum* root are shown in Table 2.

Table 2: Preliminary Phytochemical analysis of *Eranthemum nigrum* Root

Phytoconstituents	Method	Aqueous Extract	Methanolic Extract	Chloroform Extract	Pet. ether Extract
Flavonoids	Shinoda Test	-	-	-	-
	Zn. Hydrocholride test	+	+	-	-
	Lead acetate Test	+	+	-	-
Volatile oil	Stain test	-	-	-	-
Alkaloids	Wagner Test	+	+	+	-
	Hager's Test	+	+	+	-

Tannins & Phenols	$Fecl_3$ Test	-	-	-	-
	Potassium dichromate test	-	-	-	-
Saponins	Foaming Test	+	+	-	-
Steroids & Triterpenoids	Salkowski test	+	+	-	+
Carbohydrates	Molish test	+	+	-	-
Acid compounds	Litmus test	-	-	-	-
Glycoside	Keller-Killani Test	+	+	-	-
Amino acids	Ninhydrin test	+	+	-	-
Proteins	Biuret	+	+	-	-

"+" -Present " –" -Absent

Physicochemical parameters

The results attained from various determinations of physicochemical analysis are produced in Table 3.

Table 3: Physicochemical Parameters of root powder of *Eranthemum nigrum*.

Parameters	Values %w/w
Moisture content (Loss on drying)	8.25 ± 0.63
Total ash	6.56 ± 0.23
Acid insoluble ash	4.12 ± 1.22
Petroleum ether soluble extractive value	0.63 ± 0.05
Chloroform soluble extractive value	2.12 ± 0.06
Ethyl acetate soluble extractive value	6.24 ± 0.05
Alcohol soluble extractive value	8.66 ± 0.25
Water soluble extractive value	10.12 ± 0.22

Fluorescence analysis

Fluorescence analysis of root powder was performed out after treating with different solvents. Fluorescence was observed at 254 and 365 nm comparing its change of color in the visible light. The observations are presented in Table 4 shows the variation in color.

Table 4: Fluorescence analysis of *Eranthemum nigrum* root powder

Solvent used	Visible light	U.V. light	
		At short (254nm)	At long (365nm)
Distilled water	Buff	Black	Black
Methanol	Brown	black	Greenish black
1N HCl	Black	Black	Black
50% HNO_3	Black	Black	Blue

$FeCl_3$	Brownish-yellow	Dark blue	Black
$CHCl_3$	Pale green	Black	Black
Picric acid	Brownish-yellow	Dark blue	Black
Ethyl acetate	Black	Black	Greenish black

DISCUSSION

Indian systems of medicine utilize the majority of the crude drugs, which are of plant origin. It is important that standards need to be set down to control and check the identity of the plant and confirm its quality before use. Hence a detailed pharmacognostic assessment is an extremely important prerequisite. In accordance with World Health Organization (WHO), the organoleptic and histological description of a medicinal plant could be the first step towards establishing its identity and purity and should be performed before any tests tend to be undertaken[20].

E. nigrum, extensively utilized in conventional medicines, has tremendous therapeutical potential due to its various biological activities. The prominent diagnostic characteristics of root were parenchyma cells, lignified xylem vessels, Cork cells, Medullary rays with xylem vessels. These characters can be utilized for the standardization of drugs as well as useful for the preparation of plant monographs and also reduces the possibilities of adulteration. When the drug is available in the powdered form, studies of physicochemical parameters can serve as an important source to judge the purity and quality of crude drugs. Ash values are utilized to establish the quality and purity of the crude drug. It implies the existence of various impurities like carbonate, oxalate, and silicate. The water-soluble ash is a water-soluble part of total ash, employed to calculate the number of inorganic substances found in the drugs. The acid-insoluble ash comprises mostly silica and indicates contamination with earthy matter. The moisture content of drugs might be a minimum level in order to suppress the growth of microorganisms like bacteria, yeast, or fungi during storage. The extractive values are helpful to judge the chemical constituents present in the crude drug and also assist in the evaluation of particular constituents soluble in a specific solvent. Total ash and acid insoluble ash is essential indices to illustrate the quality and purity of the herbal medicine. Total ash consists of physiological ash, which is derived from plant tissue itself, and nonphysiological ash that is usually from atmosphere contaminations includes sand and soil. Total ash content alone is not adequate to indicate the quality of herbal medicine because the plant materials usually contain a significant level of physiological ash, calcium oxalate in particular. Therefore, the acid insoluble ash content is another index to indicate the quality of herbal medicine (20-22). The phytochemical analysis of extracts viz., petroleum ether, chloroform, methanol, and water were analyzed, and it indicates the presence of steroids, alkaloids, tannins, saponins, carbohydrates, glycosides, amino acids, and proteins.

CONCLUSION

Standardization of herbal drugs is very crucial because they are produced from heterogeneous sources, which could result in variations. These kinds of variations can cause spurious results in various pharmacological and phytochemical studies. *Eranthemum nigrum* roots are recognized for many therapeutical properties. Therefore, the current study might be beneficial to supplement the information with respect to its identification, authentication, and standardization; no such information is available for the same to date.

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