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Research Article

PRELIMINARY PHYTOCHEMICAL, PHARMACOGNOSTIC AND PHYSICOCHEMICAL EVALUATION OF *ERANTHEMUM NIGRUM* LEAF

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ABSTRACT

Objective: To analyze the pharmacognostic characteristics and physiochemical parameters of the leaves of *Eranthemum nigrum (E. nigrum)*.

Methods: Microscopic characters and powder analysis had been carried out with the help of a microscope. The physiochemical 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*:* Macroscopically, the leaves are simple, elliptical in shape, dull with smooth margins and acute apex. Microscopically, the leaf showed the presence of epidermal cells with uniseriate multicellular covering trichomes and diacytic stomata, followed by 4-6 layered collenchymatous cells and 10-14 numbered conjoint, collateral closed vascular bundles are some of the diagnostic characteristics observed from an anatomical study. Powder microscopy of leaf revealed the presence of uniseriate multicellular covering trichomes, lignified xylem vessels, epidermis with diacytic stomata and parenchyma cells. The investigations also included leaf surface data i.e., quantitative leaf microscopy and fluorescence analysis. Physiochemical parameters such as loss on drying, extractive values and ash values were also determined. Preliminary phytochemical screening showed the presence of steroids, alkaloids, tannins, saponins, carbohydrates, glycosides, amino acids and proteins.

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* leaves.

INTRODUCTION

Medicinal plants are usually playing a significant part in traditional medicines intended for therapy of various health issues. However a crucial hurdle, which has impeded the promotion in the usage of alternative medications in the developed countries, is lack of evidence of documentation and absence of stringent quality control measures. Additionally, there is a dependence on the data of all study 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 as well as its parts to be used like a medication. During the process of standardization, we are able to take advantage of various techniques and methodology to achieve our goal in a phase wise approach e.g. pharmacognostic and phytochemical studies.

These techniques and methods are helpful in recognition and standardization of the plant material. Appropriate characterization and quality assurance of starting material is a crucial step to ensure reproducible quality of herbal medicine to assist people in order to justify its safety and effectiveness. Because of this reason, we have executed pharmacognostic studies of *Eranthemum nigrum* belongs to family Acanthaceae (1). This sort

of research is not going to help in authentication but additionally ensures reproducibility of herbal goods in promoting (2).

In the present study, we have been focusing our exploration on one of the commonly available plant in India i.e., *Eranthemum nigrum*, belongs to 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 ethno medicinal 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 Pacific Islands. The shrub attains height 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 terminal erect 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 antiinflammatory (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 physico-chemical study is still lacking. The present investigation of *Eranthemum nigrum* (Acanthaceae) is therefore taken up to establish certain botanical and chemical standards which 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 leaf 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 air tight container for future use.

Chemicals

All analytical grade chemicals were utilized in this study were procured form 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* leaves has been carried out in accordance the colour, size, odour, 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 thin hand section of the leaf with the help of 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 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 leaf powder was extracted with 20 ml of the respective solvent by maceration at room temperature for 24 hours. Then, filtered through whatmann 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 leaf powder was blended with 1.5 ml of respective reagent (Table 4). The mixture was placed on 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* leaves were described in Figure 1 and Table 1.

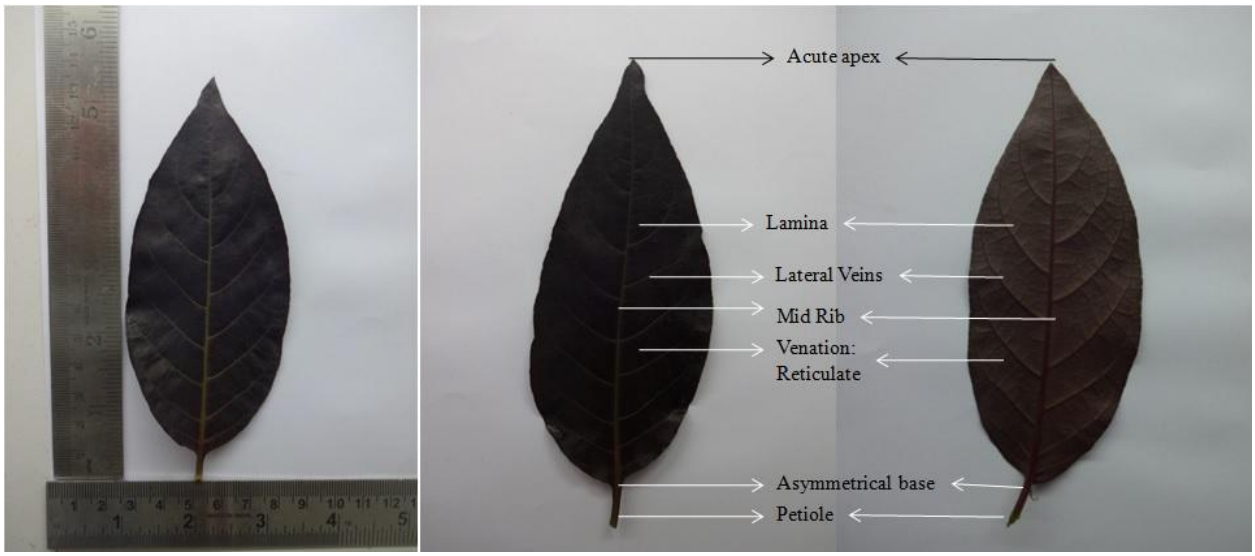

Figure 1: Morphological features of leaf of *Eranthemum nigrum*

Table1: Morphological Characteristics of Leaf of *Eranthemum nigrum*

Characters	Observation
Size	LengthL 4-13 cm (Avg); Width: 2-6 cm (Avg)
Shape	Elliptic
Apex	Acute
Base	Asymmetric
Margin	Entire
Colour	Upper Surface: Blackish purple Lower Surface: Purple
Odour	Characteristic
Taste	Characteristic
Texture	Smooth

Anatomical Description

Leaf

The transverse section of leaf passing through midrib is convexly protruding at the lower side slightly with more prominent ridged on the upper side, showed uniseriate epidermal cells on both surfaces of the leaf, which was covered by thick cuticle. The epidermis is composed of rectangular shaped cells and contains a diacytic type of stomata. There are uniseriate multicellular covering trichomes on the adaxial and abaxial surface of epidermal cells, relatively more on abaxial surface. The epidermal cells followed by 4-6 layered collenchymatous cells beneath upper epidermis and 2-3 layered collenchymatous cells above lower epidermal cells in the midrib region. The cells of collenchyma were thick walled and round in shape showing small intercellular spaces, followed by broad parenchymatous ground cells with intercellular spaces. Conjoint, collateral closed vascular bundles 10-14 were present in the ground tissue. The phloem consists of

companion cells and sieve tubes and xylem consists of spiral annular thickened vessels, tracheids, fibers and xylem parenchyma (Figure 2-6).

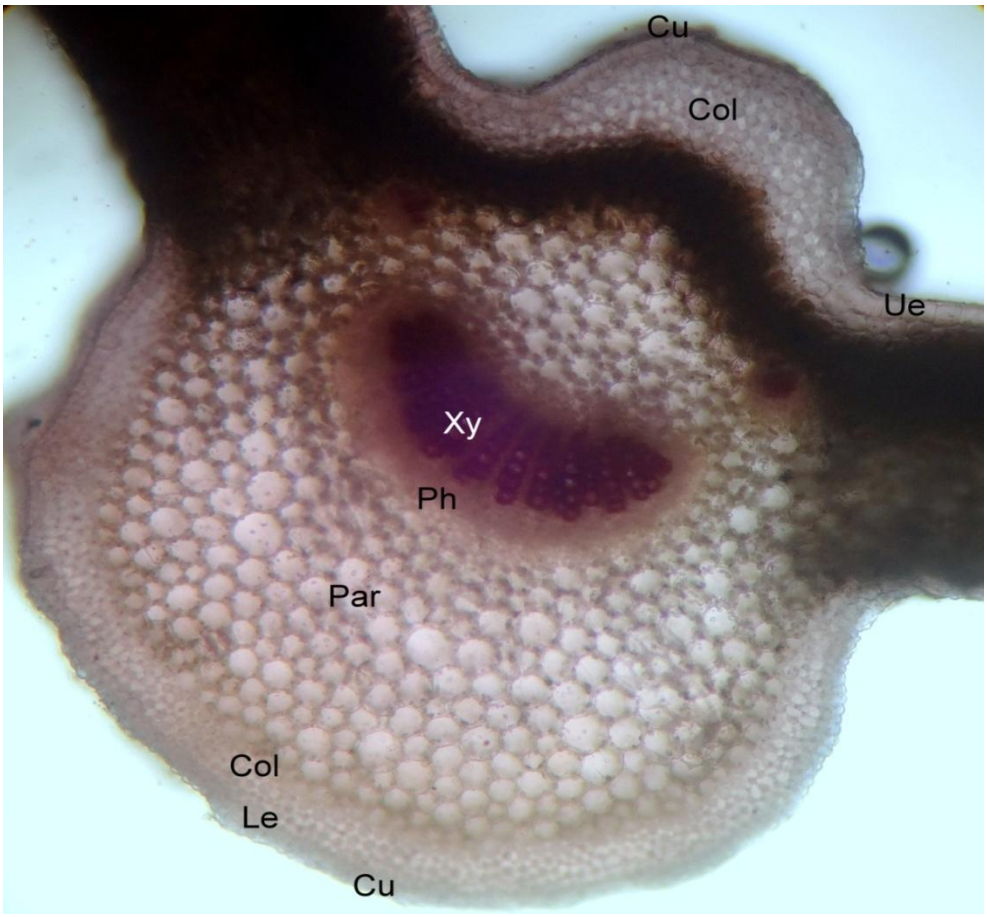

Figure 2: Transverse Section of Leaf midrib portion of *Eranthemum nigrum;* Cu: Cuticle; Ue: Upper Epidermis; Le: Lower epidermis; Col: Collenchyma; Par: Parenchyma; Xy: xylem and Ph: Phloem.

Figure 3: T.S of Midrib portion of *Eranthemum nigrum* showed Vascular Bundles Ph: Phloem; MX: Metaxylem; PX: Protoxylem.

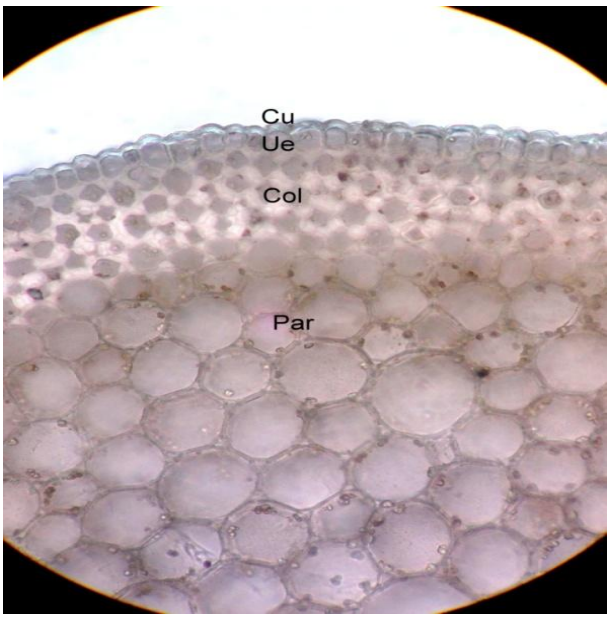

Figure 4: Detailed TS of midrib of leaf showed upper epidermis, collenchyma and parenchymatous cells of Eranthemum *nigrum*. Cu: Cuticle; Ue: Upper Epidermis; Col: Collenchyma cells; Par: Parenchyma cells.

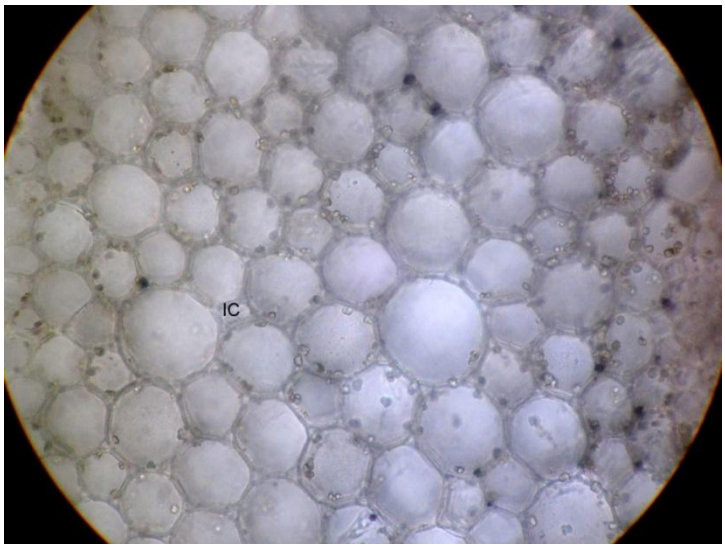

Figure 5: Ground tissue of leaf midrib showed Intercellular spaces. IC: Intercellular spaces

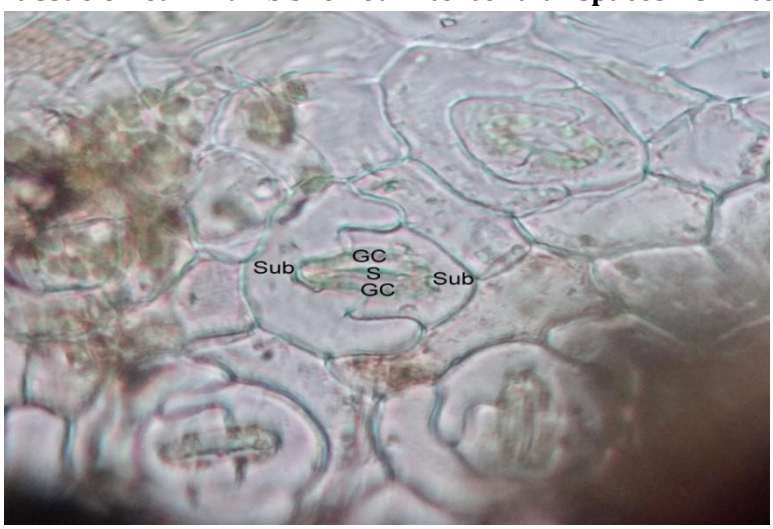

Figure 6: Epidermal cells showed Diacyticc stomata. S: Stoma; G: Guard cells; Sub: Subsidiary cells.

Petiole

Circular shaped petiole was observed in T.S, showing a layer of thickly walled epidermis with uniseriate multicellular covering trichomes. Followed by 5-7 layers of collenchymatous cells were present beneath the epidermal layer. Various sized parenchymatous cells from the ground tissue with intercellular spaces. Vascular bundles are open, bicolateral and arranged in a ring, which was present at the center of the petiole and the nature is similar to that of the leaf (Figure 7).

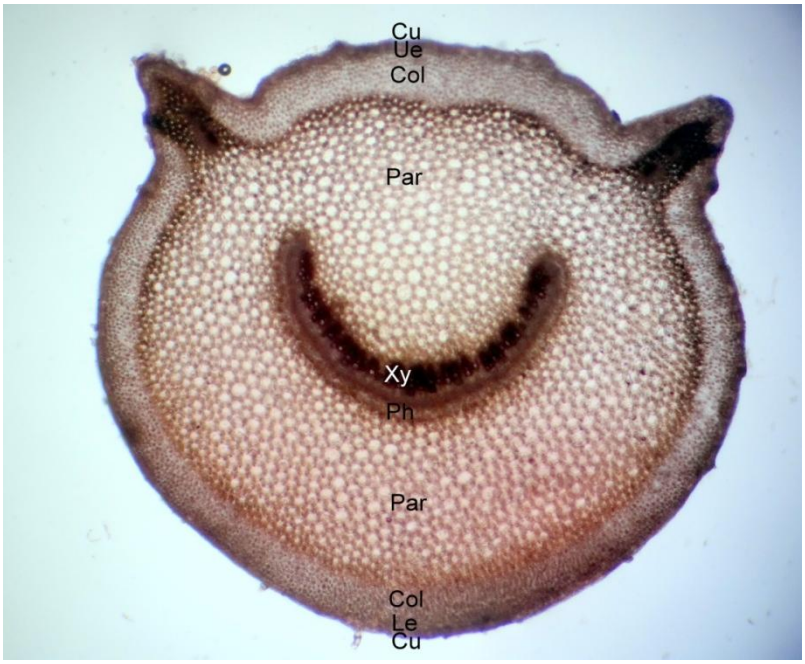

Figure 7: Transverse Section of Petiole of *Eranthemum nigrum*; Cu: Cuticle; Ue: Upper Epidermis; Col: Collenchyma; Par: Parenchyma; Xy: Xylem; Ph: Phloem and Le: Lower epidermis

Powder microscopy

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

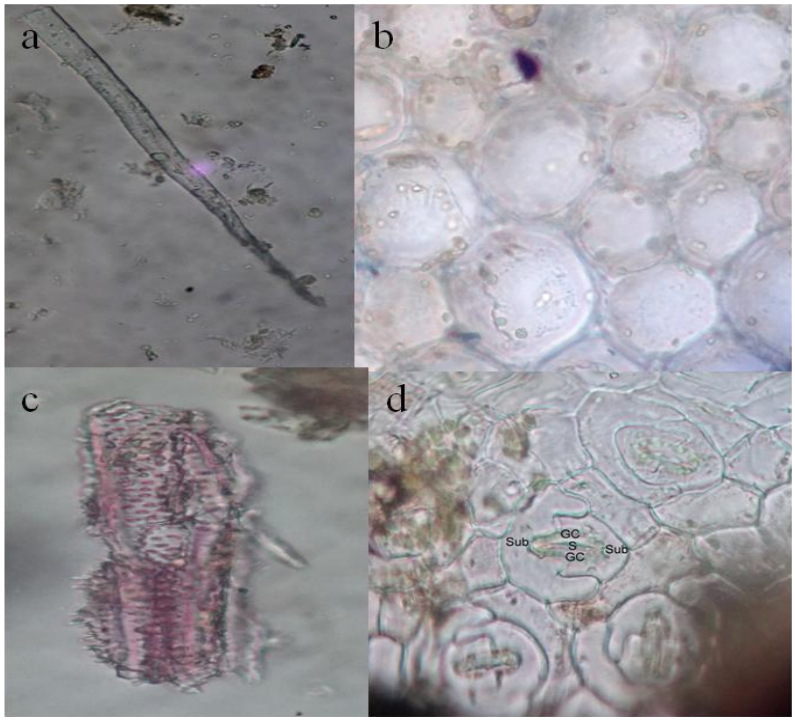

Figure 8: Powder Microscopy of Leaf of *Eranthemum nigrum* (a) uniseriate multicellular covering trichomes (b) parenchyma cells (c) lignified xylem vessels (d) epidermis with diacytic stomata

Leaf constants

Leaf venation was reticulate with 5-7 pairs of alternate lateral veins. Vein islet number is 13.4 ± 4.2 and vein termination number is 16.8 ± 3.4. The stomatal number and stomatal index for lower epidermis is 24.32±8.3 and 33.33 per sq. mm respectively, for upper epidermis 15.7 ± 6.4 and 30 per sq. mm respectively.

Preliminary phytochemical analysis

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

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

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 leaf powder of *Eranthemum nigrum*.

Parameters	Values %w/w
Moisture content (Loss on drying)	6.4±0.56
Total ash	7.84 ± 1.53
Acid insoluble ash	2.25 ± 0.76
Petroleum ether soluble extractive value	0.78 ± 0.03
Chloroform soluble extractive value	1.58 ± 0.08
Ethyl acetate soluble extractive value	4.92 ± 0.12
Alcohol soluble extractive value	9.56 ± 0.13
Water soluble extractive value	11.84 ± 0.18

Fluorescence analysis

Fluorescence analysis of leaf 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* leaf powder

Solvent used	Visible light	UV light	
		At short (254nm)	At Long (365nm)
Distilled water	Black	Black	Black
Methanol	Brown	Greenish black	Greenish black
1N HCl	Black	Black	Black
50% HNO_3	Black	Black	Blue
$FeCl_3$	Orange	Dark blue	Black
$CHCl_3$	Pale green	Black	Black
Picric acid	Purplish yellow	Dark blue	Black
Ethyl acetate	Black	Black	Greenish black

DISCUSSION

Indian systems of medicine utilize 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 extremely an 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 to 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 leaf were uniseriate multicellular covering trichomes, diacytic stomata, lignified xylem vessels and parenchymatous cells. These characters can be utilized for standardization of drugs as well as useful for preparation of plant monograph 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 water soluble part of total ash, employed to calculate the amount 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 at 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 are 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 much 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* leaves are recognized for many therapeutical properties, therefore, the current study might be beneficial to supplement the information in respective to its identification, authentication, and standardization; no such information is available for the same till date.

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