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Pharmacognostical Investigation of *ErythrinaVariegata* Linn (Fabaceae)

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Abstract

Herbal drugs constitute a major part in all the traditional systems of medicine. One of the traditionally acknowledged plant *Erythrina variegata,* commonly known as Indian coral tree belonging to the family Fabaceae has been widely used forayurvedic therapies. Due to the scarcity of standardization reports of this valuable medicinal plant the pharamacognostic, morphological, microscopical and chemical characterization studies of the leaves of *Erythrina variegata* Linn,syn were performed. Anatomical investigations of theepidermal study of leaves revealed sessile glandular trichomes on both the epidermis of the leaves. Paracytic and anisocytic stomata only on the lower epidermis of the leaf were observed.The Fluorescence analysis of leaves powder of *E. variegata* Linn,syn revealed a range of colours from dark green, yellow to brownish black under short Ultra Violet (UV) light. Various quantitative parameters like ash values, extractive values and moisture content that can be used as quality control parameters for *E. variegata* Linn,syn were determined. The air dried leaf powder methanolic extract fingerprinting pattern was developed by using High Performance Thin Layer Chromatography (HPTLC) technique which showed eight well resolved components.The pharamacognostic studies carried out for *E. variegata* Linn,syn and presented here provide referential information for the identification of this crude drug and will also help in distinguishing it from its adulterants.

Keywords

Erythrina variegata Linn, *syn, pharmacognostic studies, fluorescence analysis, HPTLC*

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INTRODUCTION

The history of herbal medicine is extremely intertwined with that of modern medicines. Many drugs listed as conventional medications were originally derived from plants. Herbal medicine is a triumph of a popular therapeutic diversity. Plants have been used as medicines from time immemorial because they have fitted the immediate personal need are easily accessible and inexpensive[1]. *Erythrina variegata* Linn, syn, is a medicinal plant widely distributed all over India. Traditionally, it is used as antihelmintic, carminative, diuretic, galactogogue, expectorant and febrifuge. It is also used to treat rheumatism and skin diseases[4].

E. variegata is a large deciduous tree armed with small conical prickles, and has stellate pubescent branches, trifoliolate leaves bearing rhomboid-ovate inequilateral leaflets 10-15 cm long, bright red flowers in clustered racemes at the tip of branches. Seeds are 4-8 in number and are compressed in the cylindrical turgid pod. This plant is commonly found in coastal areas and in the forest of the Western Ghats. The stem bark of this tree contains indole alkaloids such as erysotrine, erysodine, erysovine, erysonine, hypaphorine and N-N-dimethyl tryptophan[5-7]. The leaves also contain erysotrine, erysodine, erythraline, hypaphorine, erythrinins along with vitexin isovitexin, proanthocynins, and melilotic acid[5]**.** The red flowers yield erysotrine, erythrartine, hypaphorine, and choline[5]. In addition to fatty acids, lactins the seed also contains alkaloids similar to leaves[5].

The bark of *E. variegata* is used in therapy for anorexia, obesity, dysmenorrhoea, and skin diseases[8]. The aqueous extract of Sri Lankan *E. variegata*leaves has a sedative but not analgesic activity[9]. The leaf paste is used by some tribes to treat fresh cuts and wounds[10, 11].

According to the World Health Organization (WHO, 1998), the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials and therefore the macroscopic and microscopic studies should be carried out before any tests are undertaken[12]. Though, *E. variegata* plant is widely used for its multiple properties, there are no reports on its pharamacognostic and chromatographic standardization. Therefore, the objective of the present study is to evaluate various pharmacognostic standards like macroscopy, microscopy, ash values, extractive values,

microscopical characters of powdered leaf and development of HPTLC fingerprinting pattern for *E. variegata* powdered leaves.

MATERIALS AND METHODS

The leaves of *E. variegata*were collected from Sawale Village of Panvel district, Maharastra, India. The plant specimen was identified from Botanical Survey of India, Western Circle, Pune, India.

Microscopic analysis

For anatomical investigations, collected material was washed and fixed in Formalin–Acetic Acid–Ethyl alcohol (FAA) solution and standard microtome techniques were followed[13,14]. Transverse sections of 10 to $15x10^{-6}$ m thickness were taken and stained with Safranine-Fast green series and Toludene blue series. Photographs were taken by Leica DM 2000 microscope connected to a digital camera.

For micromorphological investigation i.e., for leaf constants, fresh as well as fixed material could be used followed by the standard peel study. Stomatal index, trichome index, palisade ratio, vein-islet and vein termination numbers were calculated by using standard method[15].

For powder study, dried plant material was finely powdered and sieved through BSS mesh No. 85. The fine powder obtained was stained using 1 % Safranine in water. The stained powder was mounted on a slide and observed under the microscope to locate and identify the characters present. The characters observed were photographed by Leica DM 2000 microscope connected to a digital camera[14].

Fluorescence analysis

This study was carried out as per the standard procedures[16]. In the present study, the plant powder was treated with 40 % aqueous sodium hydroxide and 40 % ethanolic sodium hydroxide, acids like 1N hydrochloric acid and 50% sulphuric acid[14].

Proximate analysis

The various physiochemical parameters like ash values, moisture content and extractive values content were determined by the standard methods[17,18].

HPTLC

Accurately weighed 250mg of powdered leaves were taken in a test tube. To it 5ml of methanol was added. The test tube was then placed in a test tube shaker for 90min. The extract was filtered through Whatmann filter paper no.41. The filtrate was then used for the development of HPTLC fingerprinting pattern. Ten microlitre of the sample was then accurately spotted on Silica gel 60 F_{254}

pre-coated plate with a band length of 8x10^{-3}m by Linomat V sample applicator. After sample application, the plate was developed up to 0.08m in Toluene: Chloroform: Methanol: Triethyl amine (5:5:0.5:0.2 v/v/v/v) as the mobile phase, air dried and scanned using a densitometer at 254 nm (Camag TLC scanner- 3)[14].

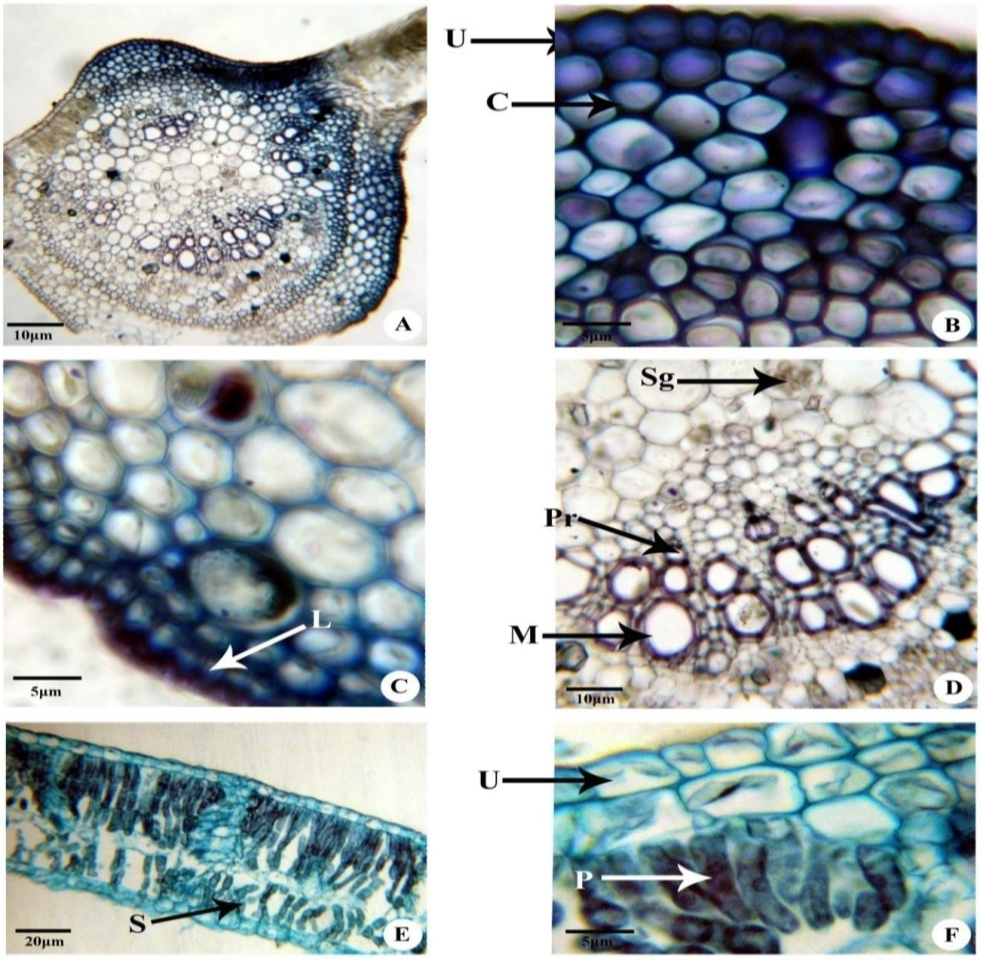

Fig 1 Microscopic images of transverse sections of leaves of *E. variegata*.

Key:

A: T.S. of Leaf
B: Upper epidermis with collenchymatous tissue
C: Lower epidermis with parenchymatous tissue
D: Midrib showing vascular bundle
E: T.S. of Lamina
F: Lamina at higher magnification Showing upper epidermis
S: Spongy tissue
U: Upper Epidermis
C: Collenchymatous tissue
L: Lower Epidermis
Sg: Starch grains
Pr: Proto xylem
M: Meta Xylem
P: Pallisade tissue

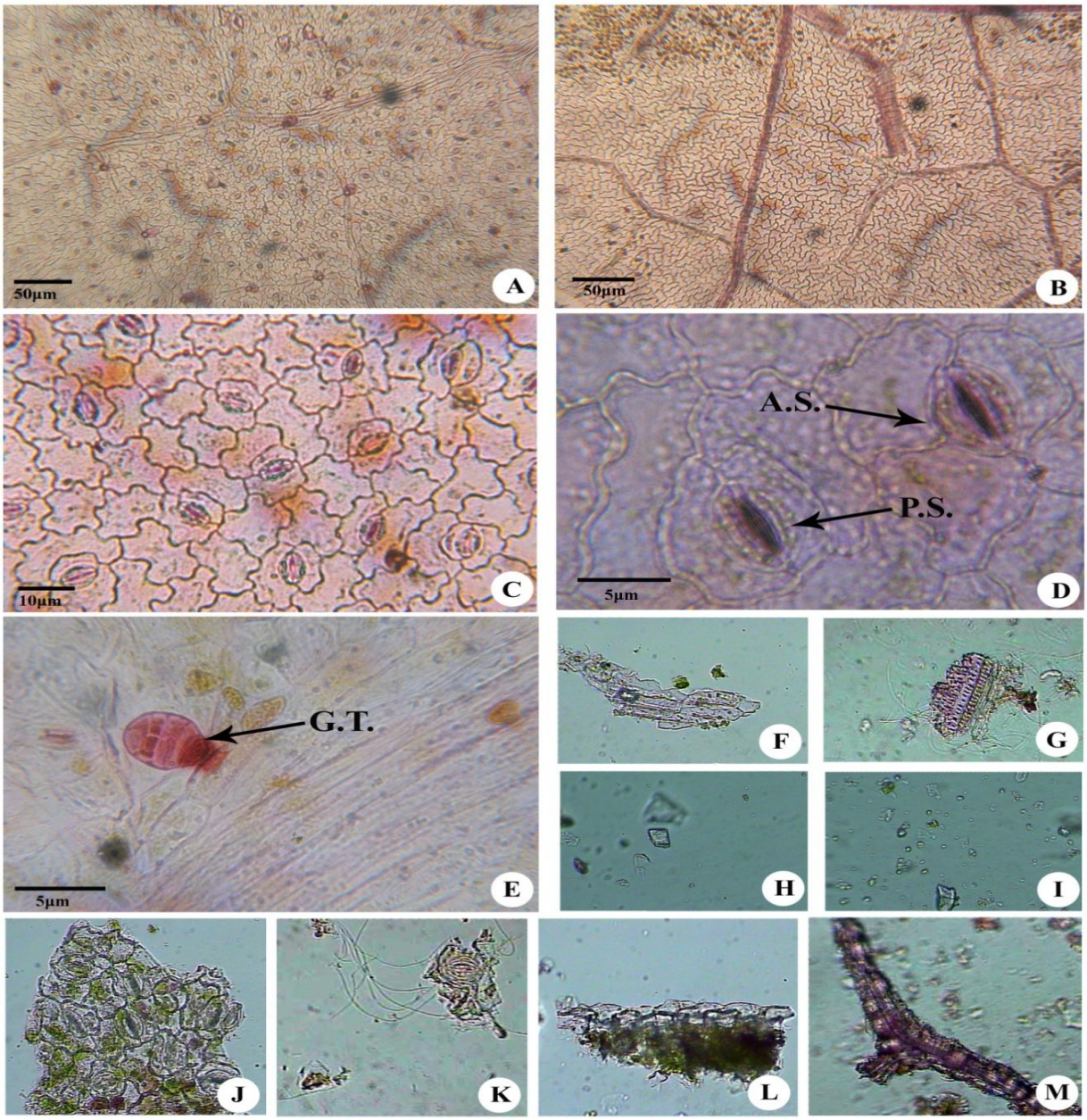

Fig 2 Micromorphological studies and powder characteristics of the leaves of *E. variegata*.

Key:

A: Lower epidermis of leaf
B: Upper epidermis of leaf
C: Lower epidermis at higher magnification
D: Epidermal peel showing two different types of stomata
E: Epidermal peel showing glandular trichome
F-M: Shows different feautures of powder studystomata

A.S.: Anisocytic stomata
P.S.: Paracytic stomata
G-T.: Glandular trichome
[F: Phloem fiber **G:** vessel with bordered pit
H: Calcium carbonate crystal **I:** Starch grain
J: Epidermis showing stomata **K:** Fibers and
L: Epidermis with palisade tissue
M: Part of midvein**]**

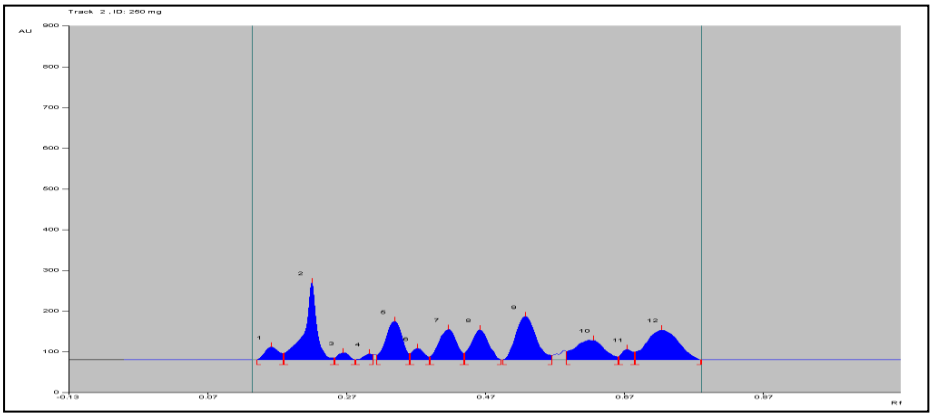

Fig. 3 This HPTLC fingerprinting pattern of methanolic extract of *E. Variegate* leaves

RESULTS AND DISCUSSION

Transverse section of leaf

A transverse section of the lamina and midrib can be observed in (Fig. 1).

The leaf was dorsiventral. Midrib region was oval (Fig, 1A) in shape and the portion of upper epidermis was projected outside and forms a crust like structure. In the midrib region, the adaxial (Upper epidermis, approx. $13x10^{-6}$m x $10x10^{-6}$m) epidermal cells were larger in size than the abaxial (Lower epidermis) epidermal cells. The epidermal cells of midrib region were rectangular to barrel in shape. In the lamina portion, the epidermal cell size was almost same at both epidermis but some variations were seen in lower epidermis. In the lower epidermis some rectangular cells were interrupted in epidermal layer. Both the epidermis were covered with cuticle. In the midrib region, the cuticle was more thickened towards the lower epidermis than the upper epidermis. The mesophyll (lamina portion) consists of three layers of elongated, compactly arranged palisade tissues (approx. $22x10^{-6}$m x $1.9x10^{-6}$m) which were present towards the upper epidermis (Fig. 1F) and 3-4 layers of isodimetric loosely arranged spongy chlorenchyma tissues with air cavities were present towards the lower epidermis. The midrib consists mainly of vascular bundles. The vascular bundles were surrounded by the parenchymatous tissue, which constitutes ground tissue. In the ground tissue, starch grains and calcium carbonate crystals were also present. At the crust portion of upper epidermis (Fig. 1B), three to four layers of collenchyma were present.

Vascular bundles consist of xylem and phloem tissues. Xylem vessels were arranged in row (Fig. 1D). The big sized vessel metaxylems were present towards the lower epidermis and small vessels known as protoxylem were present towards the upper epidermis. Phloem was present below the xylem that means towards the lower epidermis.

Determination of leaf constants

The stomatal type was decided on the basis of the arrangement of the subsidiary cells around the stomata. In *E. variegata*, subsidiary cells were distinguishable from the guard cells of the stomata. Two types of stomata were found in the epidermis of,that is paracytic and anisocytic stomata (Fig 2D). The frequency of paracytic stomata was found to be more than the anisocytic stomata. The frequency of stomatas were more on the lower epidermis and the stomatas were absent on the upper epidermis (Fig. 2A, B), hence the leaf was hypostomatic. Different types of trichomes were present on the leaves and they were differentiated based on their structures. In *E. variegata,* only glandular trichomes (Fig, 2E) were present especially on the lower epidermis. These glandular trichomes were sessile i.e. without stalk cell. These trichomes were mainly present on the main vein and also present on the minute smaller veins. The values of different leaf constants were as shown in Table 1.

Powder microscopy

The powder of *E. variegata*showed presence of abundant calcium oxalate crystal and starch grains. The plant powder also showed presence of vessels with boardedpit, simple vessel, parenchyma cells, phloem fibers, epidermal cells with stomata, palisade tissue and fibers.

The presence of two type of stomata i.e. paracytic and anisocyticwere observed which can form one of the important identifying character (Fig. 2, F-M).

Fluorescence analysis: Different colour ranges were obtained for the leaf powder in different reagents, which are given in Table 2.

Proximate analysis

The values of ash values, extractive values and moisture content are given in the Table 3.

Table 1 Calculated values of leaf constants*

S. No.	Leaf constant	*Erythrina variegata*	
1	Stomatal index	Lower epidermis 0	Upper epidermis 24.12±0.2
2	Trichome index	4.6±0.9	
3	Palisade ratio	7.8±1.3	
4	Vein iselet number	23.2±1.16	
5	Vein termination number	2-4	

*Each value is a mean of 25 readings ± S.D.

Table 2 Fluorescence Analysis

	Ordinary Light	**UV Long**	**UV Short**
Powder as such	Green	Brownish black	Dark green
Powder + 1 N HCl	Yellowish green	Dark green	Dark green
Powder + 50% Sulfuric acid	Yellowish green	Dark green	Brownish black
Powder + 40 % NaOH	Greenish yellow	Blackish green	Dark Green
Powder + 40 % NaOH-ethanolic	Dark Green	Yellowish Green	Dark Green

Table 3 Proximate Analysis

Sr. No.	**Parameters**	**% Content**
1	Total ash	5.33
2	Acid-insoluble ash	0.24
3	Water soluble ash	2.31
4	Ethanol soluble extractive	9.52
5	Water soluble extractive	22.41
6	Moisture content	8.33

Table 4 HPTLC profile of methanolic extract of *Erythrina variegata* leaves

Extract	**Solvent system used**	**Number of peaks**	**R_f values**	**Percentage peak area**
Methanolic	Toluene: chloroform: methanol: triethyl amine (5:5:0.5:0.2 v/v/v/v)	8	0.22, 0.26, 0.34, 0.37, 0.41, 0.46, 0.53, 0.72	17.11, 1.31, 10.84, 2.54, 9.41, 9.08, 15.72, 17.27

HPTLC

The suitable mobile phase, number of compounds, their R_f values and percentage peak area were determined by HPTLC Table no. 4. The chromatographic fingerprint of methanolic extract of*E. variegata* showed eight peaks Fig. 3. This HPTLC fingerprinting pattern can be considered as analytical parameter to check identity, purity and authenticity of *E. variegata*

CONCLUSION

In view of the medicinal importance of *Erythrina variegata* Linn,syn, pharmacognostic standardization has been developed for its proper identification of its

leaves. Studies for determination of leaf constants and micromorphological characteristics were performed. The results obtained from the studies like transverse section, powder analysis, leaf constants, fluorescence analysis and HPTLC fingerprint enable us to compare an authentic material with any given sample of the herb. Various quantitative parameters like ash values, extractive values, and moisture content can be set as quality control parameters confirming the identity, quality and purity of the *E. variegata* leaves. Thus, a successful attempt was made to pharmacognosticallyand chromatographically standardizes the leaves and extract of *E. variegata*. In conclusion, the data obtained in this study can be suggested asreference information for the identification of this medicinally acclaimed crude drug and also help to discern it from its adulterants.

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