

# Botanical Features for Taxonomic Evidence of *Erythroxylum monogynum* Dried Leaf

Sabeena Hussain Syed, Ajay Gajanan Namdeo\*

## ABSTRACT

**Introduction:** *Erythroxylum monogynum* belonging to family Erythroxylaceae is commonly found in southern parts of India. Traditionally, leaves of the plant possess high medicinal value and known for the treatment of diabetes, jaundice and to kill intestinal worms, which are scientifically reported. However, no elaborative pharmacognostic study of leaves has been performed yet. **Objective:** To pharmacognostically standardize the leaves by studying anatomical characters of transversal section and powdered leaves. **Material and methods:** Leaf samples were studied by macroscopy, microscopy, physicochemical and preliminary phytochemical analysis as recommended by WHO. **Results:** Externally the leaves are elliptic - lanceolate, alternate, base attenuate, apex obtuse and entire margin. Internally it shows the presence of paracytic stomata confined to lower epidermis, conjoint, collateral arc shaped vascular bundles encircled by sclerenchyma, lignified fibers and xylem vessels with spiral thickening. Powder microscopy revealed the presence of epidermis with papillae, phytochemical and physicochemical of powdered drug proved useful to differentiate the powdered drug material. **Conclusion:** The establishment of botanical features of leaf material will be useful in identification and standardization of *Erythroxylum monogynum* in obtaining quality formulations.

**Key words:** Microscopy, Paracytic stomata, Physicochemical, Phytochemical, Standardization, Vascular.

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## INTRODUCTION

Herbs represent major part in medicine for thousands of years and now herbal drugs play a significant role in health care management principally in developing countries. However, its acceptance is limited due to lack of standardization and stringent quality control. World Health Organization emphasized on standardization and quality control of herbal drugs, according to which standardization complies the physicochemical evaluation of crude drug such as ash values, moisture content, extractive values, crude fiber. The quality aspects of the drug majorly include macro and microscopic examination, qualitative and quantitative chemical evaluation and chromatographic fingerprinting.<sup>1</sup>

Correct identification and quality assurance of plant material is indispensable to ensure reproducible quality of herbal medicine, which will contribute to its safety and efficacy.<sup>2</sup> In this study, pharmacognostic standardization of *Erythroxylum monogynum* (*E.monogynum*) include its morphological, anatomical and biochemical characteristics.

*E.monogynum* Roxb belongs to family Erythroxylaceae, commonly known as Bastard sandal or Red cedar. It is commonly found in southern parts of India. In folklore medicine, parts of the plant are claimed to have medicinal benefits. The infusion of bark and wood is used as stomachic, diaphoretic, stimulant, diuretic and also in mild cases of dyspepsia and continued fever.<sup>3</sup> The leaves extract are mixed with yogurt to kill intestinal worms, where as leaf juice used for the treatment of jaundice.<sup>4,5</sup> *E.monogynum* scientifically

reported for its antibacterial activity,<sup>6</sup> hepatoprotective<sup>7</sup> and antidiabetic activity.<sup>8</sup>

The objective of the present study is to reveal the pharmacognostical, physicochemical and phytochemical aspects of leaves of *E.monogynum* which may help to develop quality, purity, authenticity of the drug and to distinguish from spurious and adulterated species

## MATERIAL AND METHODS

### Plant Material

The plant, *E.monogynum* was collected from Khammam District (Telangana) during flowering season. The plant was authenticated by Botanical Survey of India, Hyderabad. The leaves of the plant were dried, powdered and passed through 40 mesh sieve and stored in an airtight container for future use.

### Macroscopic analysis

Fresh leaves of the plant were noted for the following macroscopical character: Size and shape, color, odor, taste, surfaces, base, apex, margin, venation, lamina and presence or absence of petiole.

### Microscopical analysis

For microscopical studies, the leaves were cut and removed from the plant and thin hand sections were prepared. The sections were cleared with chloral hydrate solu-

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tion, stained with phloroglucinol-hydrochloric acid (1:1) and toluidine blue. Some hand sections were also examined in glycerine.<sup>9,10</sup> The following characteristics were observed for their presence or absence: epidermal cells, type of stomata and trichomes. The transverse sections of the fresh leaves through the lamina and the midrib are also cleared, mounted and observed.

The powdered drug of the leaves was separately treated with solution of phloroglucinol-hydrochloric acid (1:1), acetic acid and iodine solution to identify the presence of lignified fibers, calcium oxalate crystals and starch grains<sup>9</sup>.

### Physico-chemical and Florescence analysis

According to WHO guidelines parameters determined for the powdered leaves are Loss on drying, total ash, acid insoluble ash, water soluble ash. Petroleum Ether soluble extractive, Chloroform extractive value, alcohol soluble extractive (90% ethanol) and water-soluble extractive values were performed to find out the amount of ether, Chloroform, alcohol and water soluble constituents respectively.<sup>11</sup> Powdered leaf material was analyzed by treating with various organic/inorganic reagents like NaOH, HCl, HNO<sub>3</sub> and H<sub>2</sub>SO<sub>4</sub> under visible light, short ultra-violet light (254nm) and long ultra-violet light (366nm).<sup>12</sup>

### Preliminary phytochemical analysis

The air-dried leaves of *E.monogynum* were made into coarse powder. The powdered material was extracted by maceration technique using methanol as solvent for overnight. The obtained extract was filtered, evaporated to dryness and subjected to qualitative chemical tests according to standard procedures.<sup>13</sup>

## RESULTS

### Macroscopic characteristics

Macroscopically, *E.monogynum* has pinnately compound leaves, alternate in arrangement. The leaflets were 7-8 pairs and shaped obovate or oblanceolate, base attenuate or cuneate, apex obtuse, margin entire, secondary veins are pinnately arranged. The leaf size is 2-4 cm long, 0.8 to 1cm wide and petiolate where petiole is 0.3-0.6 cm in length. Fresh leaves are green, odorless and have bitter taste (Figure 1).

### Microscopical characteristics

The transverse section of a leaf of *E.monogynum* shows dorsal ventral characters. The important characteristics in the midrib regions are as follows:

The midrib slightly protrudes convexly at the dorsal side and strongly projects at the ventral side. The epidermis is composed of single layer of barrel shaped cells. Adaxial epidermis is without stomata but abaxial side consists of paracytic stomata and rounded papillae. Collenchyma consists of 2-3 layers of cells which are present below both upper and lower epidermis. Intercellular spaces are present between collenchyma cells. It is followed by cortex composed of 3 – 4 layered oval – circular parenchymatous cells. Conjoint, collateral, arc shaped vascular bundles are present which are encircled by lignified sclerenchyma fibres. Xylem vessels are lignified, composed of spiral thickening (Figure 2).

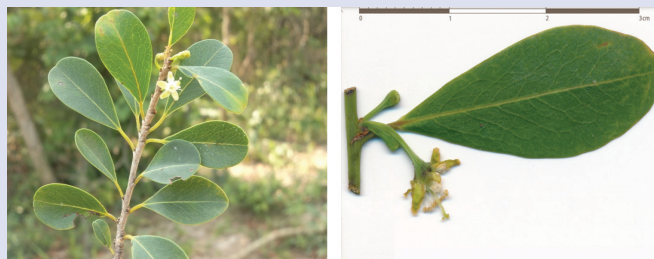
T. S. of laminar region shows both upper and lower epidermis. Palisade cells are compact, elongated and confined to lower portion of upper epidermis which is followed by loosely arranged spongy parenchymatous cells. Papillae-elongated cells are present below lower epidermis (Figure 3).

### Powder Characteristics

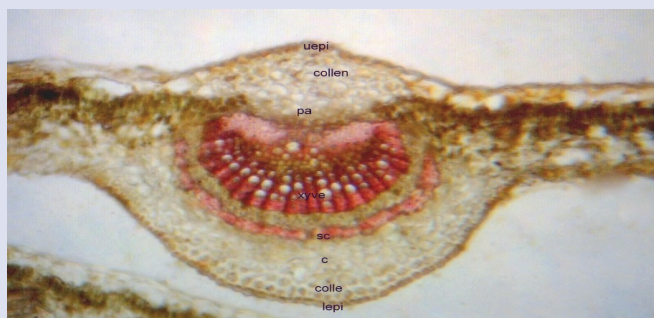
Powder is dull greenish in color, with bitter taste and faint odor. The leaf powder showed fragments of epidermis with paracytic stomata, epidermal

**Table 1: Physicochemical parameters of *E.monogynum* leaves**

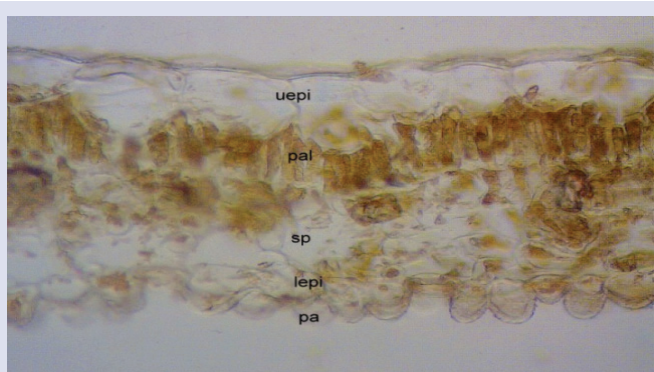
Parameters	(%w/w)
Loss on drying	6.20%
Total ash	8.03%
Acid insoluble ash	5.50%
Water soluble ash	2.51%
Pet ether soluble extractive	5.12%
Chloroform	12.15%
Alcohol soluble extractive	18.11%
Water soluble extractive	12.04%



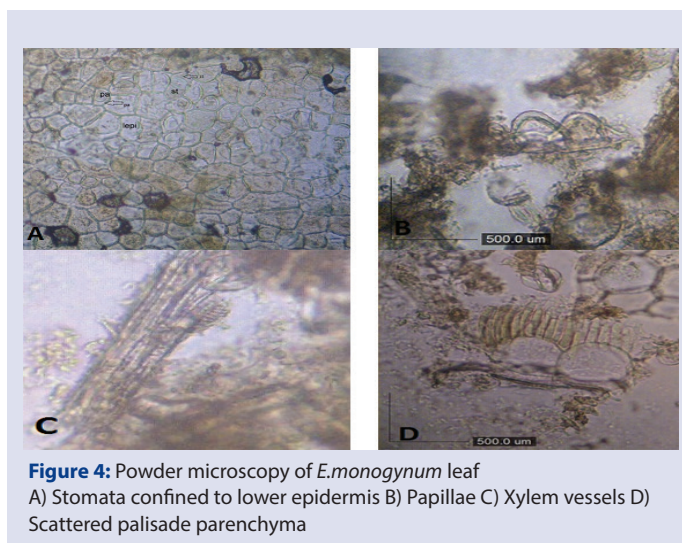
**Figure 1: Macroscopic characters of leaves of *E. monogynum***



**Figure 2: Microscopical characters of *E.monogynum* leaf midrib (T.S)**  
Uepi: upper epidermis; Collen: Collenchyma; Pa: Parenchyma; Xyve: Xylem Vessels; Sc: Sclerenchyma; C: Cortex; Colle: Collenchyma; lepi: lower epidermis



**Figure 3: T.S of laminar portion of *E.monogynum***  
Uepi: upper epidermis; Pal: palisade parenchyma; Sp: spongy parenchyma; Lepi: lower epidermis; Pa: papillae

**Table 2: Fluorescence analysis of powdered leaves of *E.monogynum***

Reagents	Color observed under day light	Color observed under ultraviolet light	
		Short (254nm)	Long (365)
Powder as such	Dark green	Green	Grey Green
1N NaOH in Methanol	Pale green	Green	Light blue
1N NaOH in water	Citrine green	Green	Light blue
1N HCl	Greenish yellow	Green	Brownish green
50% HNO <sub>3</sub>	Dark brown	Brown	Reddish brown
50% H <sub>2</sub> SO <sub>4</sub>	Brownish green	Brown	Reddish brown

**Table 3: Preliminary phytochemical analysis of *E.monogynum* leaf extract**

Chemical constituents	Chemical tests	Petroleum ether extract	Chloroform extract	Methanolic extract	Aqueous extract
Carbohydrates	Molisch's test	-	+	+	+
	Fehling's test	-	+	+	+
	Benedict's test	-	-	+	+
Alkaloids	Dragendorff's test	+	-	-	-
	Mayer's test	+	-	-	-
	Wagner's test	+	-	-	-
Saponin glycosides	Foam test	-	+	+	+
Triterpenoidal glycosides	Liebermann burchard test	+	+	+	-
	Salkowski tet	+	+	-	-
Flavonoids	Shinoda test	-	-	+	+
	Lead acetate test	-	-	+	+
Phenols	Ferric chloride test	-	-	+	+
	Bromine water test	-	-	+	+
	Lead acetate test	-	-	+	+

+: Present, -: Absent

cells with papillae in surface view. Xylem consisting of vessels with spiral thickening was observed. Fragments of sclereids and scattered palisade cells of laminar portion are the characteristic features of powder microscopy for the leaf of *E.monogynum* (Figure 4).

### Physicochemical constants

Physicochemical analysis of leaf viz. loss on drying, ash value and extractive value are presented in Table 1. The fluorescence analysis of leaf

powder observed under day light, short ultra-violet light (254nm) and long ultra-violet light (366nm) mentioned in Table 2.

### Preliminary phytochemical analysis

Qualitative analysis of leaves of *E.monogynum* petroleum extract, chloroform extract, methanolic extract and aqueous extract showed the presence of alkaloids, flavonoids, saponins, phenols, terpenoids and alkaloids in respective tests which are mentioned in Table 3



## DISCUSSION

Ethnomedicinally, the leaves of *E.monogynum* were used by local people in the treatment of some ailments without standardization. Pharmacognostic standardization plays an important role in identification, authentication and determining the level of adulteration, which help in establishing quality control of crude drugs.<sup>14</sup> In this study, pharmacognostic standards for leaves of *E.monogynum* are carried out for the first time.

The microscopical characters of the leaf which serves as diagnostic parameters, revealed the presence of conjoint, collateral, arc shaped vascular bundles containing spiral shaped xylem vessels encircled by lignified sclerenchyma fibers. The palisade parenchyma confined to lower portion of upper epidermis. Physicochemical analysis of the drug could be useful for identification of adulterants and also in compilation of a suitable monograph. Ash values are validity parameters to judge the identity or purity of the crude drug. Extractive value mainly used to determine the exhausted or adulterated drugs along with estimation of specific components soluble in particular solvents.<sup>15</sup> In this study higher extractive value of methanol than water indicates more methanolic soluble components. The fluorescence analysis of drug extract helps to identify the drug with specific fluorescent colors and to resolve doubtful specimens.

Phytochemical evaluation is to determine the class of compounds present in the plant parts, which also suggests possibility of any potent pharmacological active constituents in determination of biological assays.<sup>ss16</sup>

## CONCLUSION

The present study established pharmacognostic standardization of leaves of *E.monogynum*, which can serve as an important source to determine the quality and purity of the drug. This work also could be useful in compilation of a suitable monograph and further establishing standard data in pharmaceutical preparation from leaves of *E.monogynum*.

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## CONFLICT OF INTEREST

None

## ABBREVIATION USED

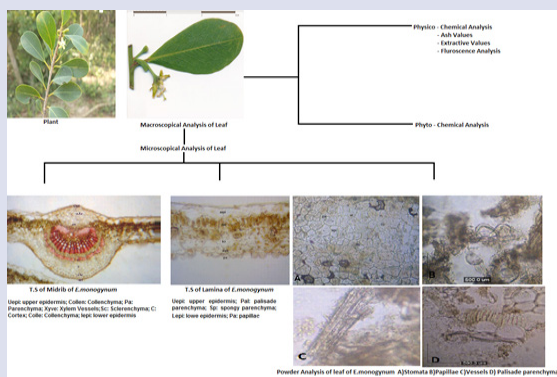
TS: Transverse section; *E. monogynum*: *Erythroxylum monogynum*;

WHO: World Health Organization;

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## GRAPHICAL ABSTRACT



## SUMMARY

- The plant *Erythroxylum monogynum* is claimed to have medicinal benefits in folklore medicine.
- Microscopical characters of leaves revealed the presence of paracytic stomata, collenchyma in the midrib region and presence of Conjoint, collateral, arc shaped vascular bundles encircled by lignified sclerenchyma fibres.
- Phytochemical analysis showed the presence of alkaloids, flavonoids, saponins, terpenoids and phenols.
- Pharmacognostical evaluation of leaves of *Erythroxylum monogynum* could be useful in standardization and in compilation of suitable monograph.

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