Pharmacognostic Parameters of *Eucalyptus globulus* Leaves

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ABSTRACT

Introduction: *Eucalyptus globulus* Labill synonym blue gum tree (Family: Myrtaceae) is an evergreen tree, one of the most widely cultivated trees native to Australia, South Africa, India and Southern Europe. The bark was used against toothaches and gum aches.The leaves were used against headaches which occured on one side of the head. *Eucalyptus globulus* Labill synonym blue gum tree (Family: Myrtaceae) possess antidiabetic, anti-inflammatory, antibacterial, antimalarial and Antioxidant properties. **Methods:** The present study will assist in standardization parameters like morphological characters, microscopic evaluation, physicochemical evaluations (loss on drying, ash values, extractive values), preliminary phytochemical screening and TLC chromatographic profile of the extract were carried out and the quantitative parameters were reported. **Results:** Chief macroscopic and microscopic characters include lamina with secretory cavity. Lower palisade cells, Lateral vein, secretory cavity, Spongy mesophyll, Upper palisade, stomata and with terminal clusters of sclereids. **Conclusion:** These studies provide referential information for correct identification and standardization of this plant material.

Keywords: *Eucalyptus globulus,* standardization, microscopic evaluation, TLC.

INTRODUCTION

The World Health Organization estimates that 80% of the world's population relies on herbal medicine. Meanwhile, the use of herbs in the United States is expanding rapidly, to the point where herbal products are readily found in most pharmacies and supermarkets. From 1990 to 1997, as the use of complementary and alternative medicine rose from 34 to 42%, herbal use quadrupled from 3 to 12%. It is worth remembering that these rapid changes have come not through the medical profession, but by popular demand. The public has discovered that natural medicines often provide a safe, effective, and economical alternative to pharmaceuticals, and research validates this finding. The majority of those who use herbal and high-dose vitamin products fail to tell their physicians. Either they assume that these products are harmless and not worth mentioning or they fear being ridiculed by doctors skeptical about their use. These same doctors, however, must begin to familiarize themselves with the subject. Aside from the advantages of the natural products, herb– drug interactions are a growing concern: almost one in five prescription drug users also using supplements.[1] India has a rich heritage of traditional medicines and the traditional health care systems have been flourishing for many centuries. It mainly consist of three major systems namely Ayurveda, Siddha and Unani systems of Medicine.[2] In almost all the traditional systems of medicine, the quality control aspect has been considered from its inspection itself by the Rishis and

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later by the Vaidya and Hakims. However, in modern concept it requires necessary changes in their approach. Quality control and quality assurance is an integral part of traditional medicines, which ensures that it delivers the required quantity of quality medicament.[3]

Eucalyptus globulus Labill synonym blue gum tree (Family: Myrtaceae) is an evergreen tree, one of the most widely cultivated trees native to Australia, South Africa, India and Southern Europe. The bark was used against toothaches and gum aches. It was crushed and placed on the area of the pain. The leaves were used against headaches which occured on one side of the head. It was claimed that the juice from the leaves was squeezed into both the nostrils. The plant also reported for antibacterial,[4] antidiabetic,[6] antioxidative,[7] antiplaque[8] antiviral,[9] anti-inflammatory,[10] and antifungal activities.[11]

Quercetin, d-limonene, dipentene, citronellal, caffeic, gentisic and protoca techuic acids are some of the compounds isolated from the *Eucalyptus globulus* Labill leaves.[12–15] The traditional use against headaches may be explained by these compounds as they possess analgesic, anti-inflammatory and antianxiety properties.[16] However no scientific standards or pharmacognostic parameters are yet available to determine the quality of this crude drug. Thus the present study was designed to evaluate the pharmacognostic parameters of *Eucalyptus globulus* Labill leaves.

MATERIALS AND METHODS

Collection and authentication

Eucalyptus globulus Labill leaves were collected in the month of February 2006, from university campus of Punjabi University, Patiala, India. The taxonomic identity of the plant was confirmed by Dr. H.B. Singh, Head, Raw Materials Herbarium & Museum, National Institute of Science Communication and Information Resources (CSIR), New Delhi 110067. A voucher specimen no NISCAIR/RHM/F-3/2006/Conslt/655/136 has been deposited in same herbarium.

Phytochemical screening

The various extracts of *Eucalyptus globulus* Labill were subjected to qualitative chemical examination.[17,18]

Thin layer chromatographic profile

TLC glass plates (5 × 15 cm), 0.25 mm thick were prepared using silica gel G. The plates were activated at 110°C for 30 minutes. The TLC profiles of the extracts were studied using different solvent systems. TLC plates were developed in TLC chamber. Thin layer chromatograms were visualized under 254/366 nm UV light and in iodine chamber. Spraying reagent 5% methanolic-sulphuric acid is used.

Organoleptic evaluation

Organoleptic evaluation of leaves was done by observing fruits and seeds with naked eyes.

Microscopic and histological techniques of leaves

Study of transverse sections

The leaves of *Eucalyptus globulus* Labill were boiled with water until soft. Free hand sections of both fruits and seeds were cut transferred on slides cleared by warming with chloral hydrate and mounted in glycerin. The lignified and cellulosic tissues were distinguished using differential staining techniques.[19]

Photomicrography

Microscopic evaluation of tissues was supplemented with micrographs. Photographs of different magnifications were taken with Nikon Labpot 2 microscopic unit. For normal observations bright field was used. For the study of crystals, starch grain and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars.[20]

Powder microscopy

A few drops of chloral hydrate solution was added to a sample of powered plant material on a slide, covered with a glass slip and heated gently over a microbunsen. Vigorous boiling was avoided. The slide was examined under the microscope. When the clearing process is completed a drop of glycerol solution was added which will prevent crystallization of the mounting agent on cooling.

Physicochemical parameters

Physicochemical analysis i.e., alcohol (90% ethanol) and water soluble extractive values, total ash, acid-insoluble ash, and loss on drying of the powdered drug were determined.[21, 22]

Quantitative microscopy

Numbers of leaf measurements were used to study microscopic features not easily characterized by general

microscopy. These included-Stomatal Number Vein Islet number-Veinlet termination number-Stomatal Index, Palisade ratio-The average number of palisade cells beneath each upper epidermal cell is termed the palisade ratio.[19]

RESULTS

Organoleptic features of leaves (Figure 1).

Condition	Fresh adult leaves
Colour	Dark green
Odour	Characteristic
Shape	Lanceolate
Dimensions	Length-10-15 cm, Width-2–4 cm
Leaf base	Exstipulate
Margin	Entire
Apex	Acuminate
Base	Symmetrical
Surface	Glabrous
Texture	Coriaceous
Venation	Pinnate lateral veins anastomose near the margin to a continuous line
Petiole	Short and twisted

Microscopic features of the leaves

The lamina has smooth and even surfaces. It is 220 µm thick. It has distinct adaxial and abaxial epidermal layers of small, squarish, or rectangular cells with prominent cuticle. These were wide, circular, empty secretory cavities in the upper part of lamina; these cavities were 70–90 µm in diameter. The cavity has this thin layer of epithelial cells. The mesophyll consists of 6 or 7 layers of palisade parenchyma cells. These cells were normally cylindrical, short, and compact. A long middle of the mesophyll zone, one or two layers of lobed loosely spongy parenchyma cells may be seen. The marginal part has thick walled, angular, compact parenchymatous tissue (Fig. 2 and Fig. 3). The lamina has dense reticulate veins and veinlets (Fig. 4). The primary and secondary veins branch profusely and give rise to ultimate veinlets. The vein islets were distinct, small and squarish or rectangular. Each vein islet has one or two vein terminations. The terminations were unbranched or branched once (Fig. 5). The vein-terminations have a cluster of large, lobed sclereids called terminal sclereids (Fig. 6).

The anatomical feature of the leaf shows that it was xenomorphic, isobilateral, smooth and even. (Fig. 7 and Fig. 8). The lamina and midrib were less differentiated from each other. Midrib (Fig. 7), is 370 µm thick, it has epidermal layer of small hemispherical cells with heavy cuticle. The vascular bundle of the midrib was single, large and

Figure 1. Organoleptic features of leaves.

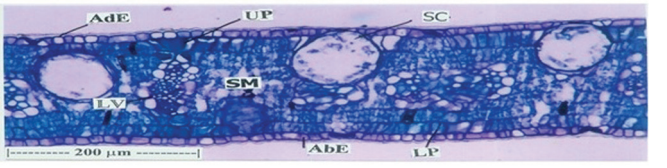

Figure 2. TS of lamina with secretory cavity. [AbE- Abaxial epidermis, AdE- Adaxial epidermis, LP- Lower palisade cells, Lv- Lateral vein, SC- secretory cavity, SM- Spongy mesophyll, UP- Upper palisade cells].

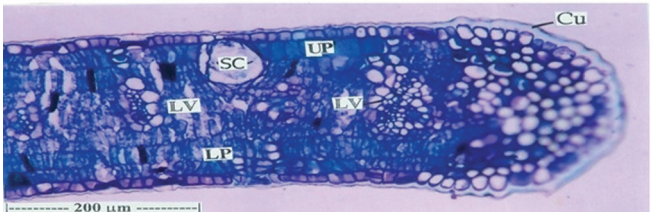

Figure 3. TS of leaf margin. [LP- Lower palisade cells, Lv- Lateral vein, SC- secretory cavity, SM- Spongy mesophyll, Cu- Cuticle, UP- Upper palisade cells].

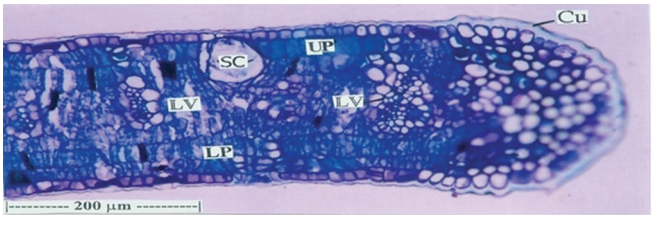

Figure 4. Venation pattern in the surface view. [Lv- Lateral vein, VI- Vein islets, VT- Vein termination].

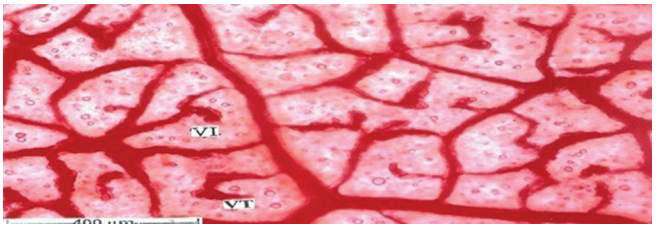

Figure 5. Vein-islets and Vein-termination. [VI- Vein islets, VT- Vein termination].

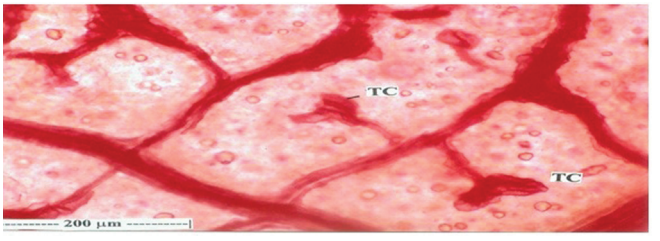

Figure 6. Vein-termination with terminal clusters of sclereids. [TC- Terminal clusters of sclereids].

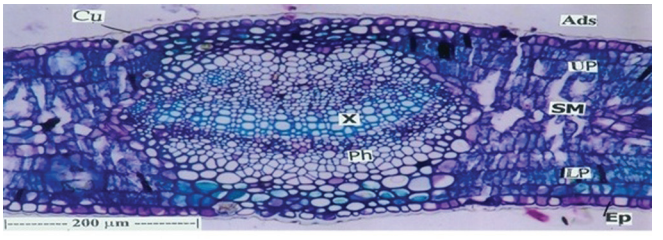

Figure 7. TS of leaf through midrib with lamina. [Ads- Adaxial side, Cu- Cuticle, Ep- Epidermis, LP- Lower palisade cells, Ph- phloem, SM- spongy mesophyll, UP- Upper palisade cells].

elliptical comprising of a wide horizontal band of xylem and a broad zone of phloem. The xylem consists of small, thick walled vessels and fibres. The vascular bundle was surrounded by wide parenchyma zone both on the adaxial and abaxial sides. In between the epidermal layers and were seen four or five layers of thick walled compact parenchyma cells. The primary lateral vein was also large and hop-shaped (Fig. 8). It has horizontal band of many xylem elements which form radial parallel rows. Phloem occurs in a thin band beneath xylem. The vascular bundle has thick sclerenchyma sheath both on the adaxial and abaxial sides. The lateral vein was 250 µm thick. The small veinlets have circular vascular bundles with small clusters of xylem and phloem, surrounded by parenchyma bundle-sheath. The vascular bundles of the lateral vein were situated in the median part of the mesophyll tissues.

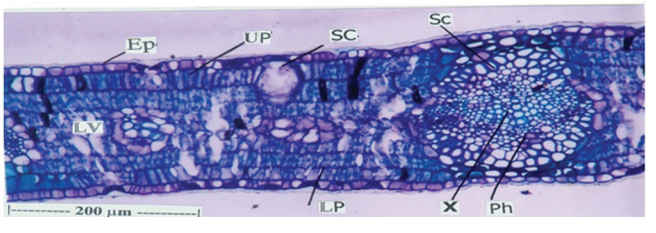

Figure 8. TS of leaf through lateral vein with lamina. [Ep- Epidermis, LP- Lower palisade cells, Ph- phloem, Sc- Sclerenchyma, SC- Secretory cavity, SM- spongy mesophyll, UP- Upper palisade cells.

The stomata were cyclocytic type. Each stoma was surrounded by 5 or 6 subsidiary cells. The guard cells were 25–30 × 20–22 µm in size. The epidermal cells were polyhedral with straight anticlinal walls (Fig. 9). Calcium oxalate crystals were fairly abundant in the leaf mesophyll. They are druses which are spherical spiny druses were seen in the mesophyll (Fig. 10).

Powder microscopy

Broken pieces of lamina were seen in the powder. They show the vein-islets which were four or five angled, large and distinct (Fig. 11), the broadening veins were thick and consist of fibres. Fibres were abundant in the powder; they were long, thick walled and lignified. The fibers were either straight or bent at one end or both ends (Fig. 12). They range in length from 500–800 µm.

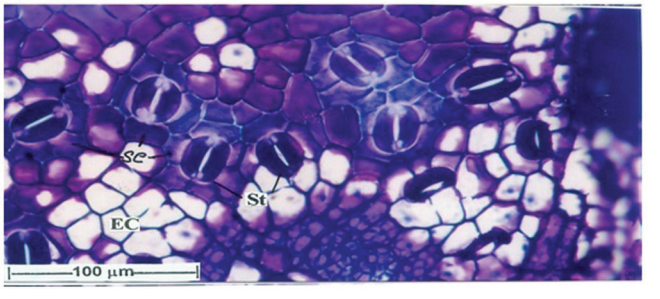

Figure 9. Abaxial epidermis with stomata. [EC- Epidermal cells, Sc- Subsidiary cells, SC- Secretory cavity, St- Stomata, V- Vein].

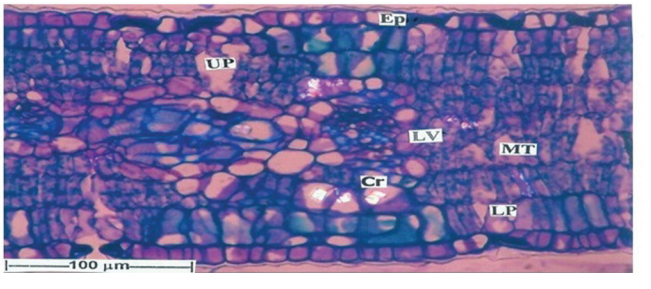

Figure 10. Druses Crystal distribution in the mesophyll tissue. [Cr- crystals, Ep- Epidermis, LP- Lower palisade cells, LV- Lateral vein, MT- Mesophyll tissue, UP- Upper palisade cells].

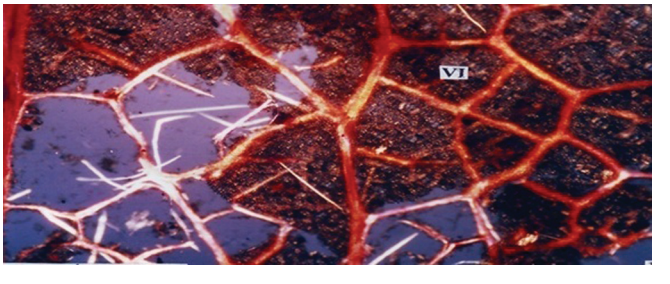

Figure 11. A fragment of lamina showing distinct vein-islets. [VI- Vein islets].

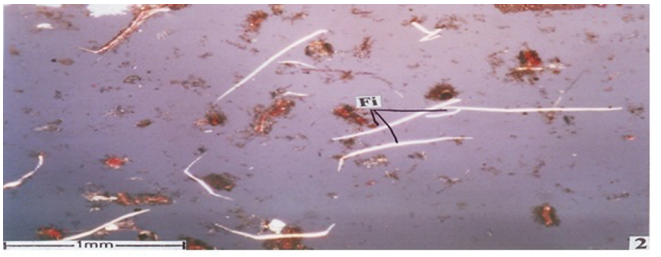

Figure 12. Fibres of the veins (under polarized light microscope). [Fi- Fibres].

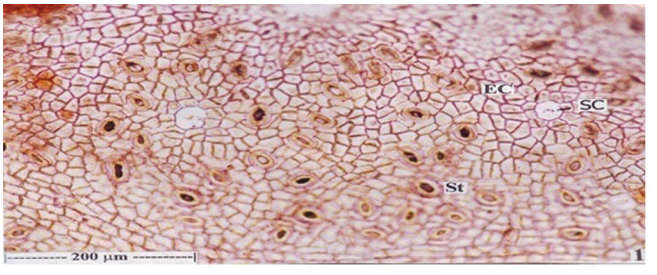

Figure 13. Powder of the leaf showing epidermal morphology. [Epidermal cells, St- Stomata].

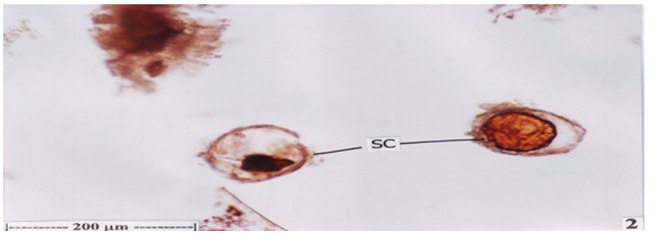

Figure 14. Oil bodies. [SC- Secretory cells].

Epidermal cells and stomata were seen in small broken pieces of epidermis (Fig. 13). The epidermal cells were small and polyhedral in shape. The anticlinal walls of the cells were thick and straight. Secretory cavities were sporadically seen in the epidermis. Stomata were abundant on both epidermal layers. The stomata were anomocytic type (Fig. 13). Some of the stomata were large measuring 30 × 40 µm; others were smaller measuring 20 × 30 µm. Most of the layered stomata have closed, dark stomatal aperture. The powder contains large spherical cells containing dark oil bodies were frequently seen in the powder (Fig. 14). The oil containing cells were 100 µm in diameter.

Physical parameters

The physical parameters of powdered leaves of *Eucalyptus globulus* Labill were evaluated as shown in (Table 1).

Table 1. Physical parameters of leaves of *Eucalyptus globulus* Labill.

Physical parameter	% (with reference to air dried drug)
Ethanol-soluble extractive	4.1
Water soluble extractive	10.5
Total ash	14.2
Acid insoluble ash	6.5
Loss on drying	23.4

Thin layer chromatography

The TLC plates of various extracts of *Eucalyptus globulus* leaves were visualized using UV, Iodine Chambers. The Rf value of various extracts in the different mobile phase was as follows (Table 2).

Preliminary phytochemical screening

The preliminary phytochemical investigation of the Petroleum ether, chloroform extracts, methanol and aqueous extracts of *Eucalyptus globulus* Labill leaves shows the presence of carbohydrates, steroids, flavonoids, phenolic compounds and tannins (Table 3).

Table 2. TLC Chromatographic profile.

Extracts	Mobile phase	Rf Values
Pet. Ether Ext.	P.E: Toluene: Ethyl acetate (3:1:1)	0.256, 0.464, 0.648, 0.808, 0.976
$CHCl_3$ Ext.	Toluene: Ethyl acetate: Acetone (7:2:1)	0.45, 0.56, 0.89, 0.96
MeOH Ext.	Chloroform: Methanol (3:2)	0.75, 0.99
Aqueous Ext.	Chloroform: Methanol (8:2)	0.99, 0.515, 0.442

Table 3. Phytochemical screening of the extracts of the *Eucalyptus globulus* Labill leaves.

Phyto-chemicals	Petroleum Ether Extract	Chloroform Extract	Methanol Extract	Aqueous Extract
Alkaloids	–	–	–	–
Carbohydrate	+	+	+	+
Protiens & amino acids	–	–	–	–
Phytosterols	–	+	+	–
Phenolic compounds and tannins	–	–	+	+
Saponins	–	–	+	+
Triterpenoids	–	+	+	–
Flavonoids	–	–	+	–

Table 4. Leaf constants of *Eucalyptus globulus.*

Stomatal Number	Upper Epidermis = 180-200-225 Lower Epidermis = 194-224-266
Vein Islet number	Range- 15.3–21.7, Average- 18.67
Veinlet termination number	Range- 18.8–23.5, Average- 20.3
Stomatal Index	Upper Epidermis- 3.8-4.4-5.9 Lower Epidermis- 9.4-10.5-11.7
Palisade ratio	Upper Epidermis- 5.5-6.4-6.9 Lower Epidermis- 4.2-5.5-6.3

Quantitative microscopy (Table 4).

DISCUSSION

As a part of standardization, the macroscopical examination of leaves of *Eucalyptus globulus* was studied. Macroscopical evaluation is a technique of qualitative evaluation based on the study of morphological and sensory profiles of drugs. The macroscopical characters of the fruits of plant can serve as diagnostic parameters. The microscopic evaluation of leaves of *Eucalyptus globulus* and extractive values, ash values and loss on drying of the powdered drug and phytochemical screening of the extract have been carried out which would be of considerable use in the identification of this drug. Percentages of the extractive values, ash value and loss on drying were calculated with reference to the air-dried drug. The percent extractives in different solvents indicate the quantity and nature of constituents in the extracts. The extractive values are also helpful in estimation of specific constituents soluble in particular solvent. Thin layer chromatography (TLC) was examined in short UV (254 nm) and long UV (366 nm) which is particularly valuable for the preliminary separation and determination of plant constituents. Numbers of leaf measurements were used to study microscopic features not easily characterized by general microscopy. This finding is useful to supplement the existing information with regard to identification and standardization of *Eucalyptus globulus* leaves even in the powdered form of the plant drug to distinguish it from drug and adulterant. These studies also suggest that the observed pharmacognostic and physiochemical parameters are of great value in the quality control and formulation development.

CONCLUSION

The present study may be useful to supplement the information with regard to its standardization and identification and in carrying out further research and its use in Ayurvedic system of medicine.

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