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Original Article

PHARMACOGNOSTIC STUDY OF *CALLISTEMON CITRINUS* L. BARK

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ABSTRACT

Objective: Pharmacognostic study of *Callistemon citrinus* L. bark

Methods: The bark of *Callistemon citrinus* L. was shade dried and powdered mechanically. The powdered material was used for further Pharmacognostic study. The study was conducted as per the guidelines of the World Health Organization which includes macroscopy, microscopy, physicochemical, Phytochemical and fluorescence studies.

Results: The bark is greenish gray and exfoliating or striated with splintery and granular fracture. Microscopy showed the presence of rhytidoma with lignified sclerenchyma tissue, abundant lignified phloem fibers and uni to biseriate medullary rays. The total ash, acid insoluble ash, water soluble ash values was found to be 7.5%, 17%, 2% w/w respectively. Moisture content was not more than 1%, methanol extractive value was 12% w/w and foreign organic matter was 1.5% w/w. The preliminary phytochemical studies showed the presence of alkaloids, flavonoids, steroids, triterpenoids and carbohydrates.

Conclusions: The results of the study could be useful in setting quality parameters for the identification of crude drug and preparation of a monograph.

Keywords: *Callistemon citrinus* L., physicochemical, quantitative.

INTRODUCTION

Plants and plant derived products have been utilized as a source of medicine for the treatment of various diseases from the advent of human civilization. Various traditional systems of medicine describe the role of plants in traditional health care. Plants are a reservoir of potentially useful chemical compounds which served as drugs and provides newer lead molecules for modern design and synthesis [1-2]. *Callistemon citrinus* L., commonly known as 'red bottle brush', is one of those medicinal plants with great medicinal importance. The name of the plant, *Callistemon,* is derived from Greek *kalos* meaning beautiful and *stemon* meaning stamens and *citrinus* from Latin *citrinus* meaning lemon, referring to the scent of the leaves [3]. It is an beautiful evergreen tree belonging to the family Myrtaceae. It is indigenous to Queensland and New South Wales and cultivated throughout India in gardens. It is sometimes considered a synonym of *Melaleuca*. The plant is commonly named as bottle brush because the cylindrical brush like flowers resembles traditional bottle brush [4]. Different parts of the plant are used by rural people of India. The plant is known in folk medicine for its anticough, antibronchitis, insecticidal effects and its volatile oil has been used as antimicrobial and antifungal agents [5-8]. Moreover aerial parts of *Callistemon citrinus* are practiced traditionally in ethnic tribal communities and very little are known about its importance on scientific grounds [9]. The leaves of *Callistemon citrinus* were evergreen, aromatic, alternate, lanceolate with entire margin and anomocytic stomata. Stem was grey in colour. Phytochemical study on leaves reported the presence of flavonoids, alkaloids, terpenoids and steroids [9]. As there is no record of research work available on pharmacogenetics parameters of the bark of the selected plant the present study was conducted to explore the anatomical features which will help in the identification and standardization of *Callistemon citrinus* L. The results will help in the preparation of the monograph.

MATERIALS AND METHODS

Plant material

The plant material was obtained from Bhimavaram of East Godavari District and authenticated by P. Prasanna Kumari, Department of Botany, DNR College, Bhimavaram; a specimen is preserved in the college herbarium of Shri Vishnu College of Pharmacy (Voucher number: SVCP/Cognosy/5).

Macroscopy

The macroscopic features of the fresh bark of *Callistemon citrinus* L. were determined using the method of Evans [10].

Microscopy

Fresh parts of the plant were cut and fixed in a mixture of formalin (5 ml) + acetic acid (5 ml) + 70% ethyl alcohol (90 ml). After 24 hrs the specimens were dehydrated with graded series of tertiary-butyl alcohol (TBA) [11]. Paraffin wax (melting point 58-60°C) was gradually added to the specimens. The specimens were cast into paraffin blocks**.** The paraffin embedded specimens were sectioned with the help of rotary microtome. Later the sections were freed from the wax. Three sections were taken i. e. Transverse section, radial longitudinal section and tangential longitudinal section. The sections and powdered samples were stained with reagents like phloglucinol and hydrochloric acid and finally mounted in glycerin for the study of microscopical characters. The study was carried out according to the methods outlined by Brain and Turner [12-13]. Different cell components were studied as per the standard methods [14] and their photographs were taken using photomicrography.

Physicochemical and phytochemical analysis

Physicochemical parameters such as ash values, moisture content and extractive values were determined according to the well-established official method and procedure [15-18]. Preliminary phytochemical screening was carried out using the standard procedure described by Khandelwal [17, 19].

Fluorescence analysis

When the sample is exposed to ultraviolet radiation many crude drugs exhibit the fluorescence. Evaluation of crude drugs based on fluorescence in daylight is not much used, as it is usually unreliable due to the weakness of the fluorescence effect. Fluorescence lamps eliminate visible radiation from the lamp as they are fitted with suitable filters and transmit ultraviolet radiation of the definite

wavelength. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives by applying different reagents. Hence some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation. The changes in appearance and colour were observed and recorded. Powdered plant material was treated with various chemical reagents and exposed to visible and ultraviolet (UV) light to study their fluorescence behavior [20].

RESULTS

Taxonomic classification

Kingdom- Plantae; Subkingdom – Tracheobionata; Division – Magnoliophyta; Class – Magnoliophyta; Order – Myrtales; Family – Myrtaceae; Genus – *Callistemon*; Species- *citrinus.*

Synonyms

Melaleuca citrina (Curtis) Dum. Cours., Callistemon lanceolatus (Sw.) DC., Callistemon laevis, Melaleuca citrine, Metrosideros citrine. Common name

Crimson Bottle Brush, Red Bottle Brush, Lemon Bottle Brush.

Tradition uses

A natural herbicide, Mesotrione, was produced by the roots of *Callistemon citrinus* L. [21]. The leaves are used as a tea substitute and have a delightfully refreshing flavor [22]. A cinnamon tan dye is obtained from the flowers [23]. Wood is hard, heavy, tough and used for tool handles [24]. It is also used for fuel [25].

Macroscopy

Callistemon citrinus L. is a handsome shrub or a small tree, up to 7.5 m in height, indigenous to Queensland and New South Wales, frequently cultivated throughout India in gardens. Leaves are lanceolate, alternate about 1 cm in length. Flowers are crimson with dark red anthers and 10 cm long spikes; capsules depressed-globose. Each flower head produces a profusion of triple-celled seed capsules around a stem which remains on the plant with the seeds enclosed until stimulated to open when the plant dies or fire causes the release of the seeds. Bark is light green or light gray, exfoliating or striated (fig. 1), with splintery fracture [26]. Bark is tasteless and odourless.

Microscopical characters

Transverse section (fig. 2) of the bark showed the presence of periderm, rhytidoma, primary and secondary phloem, cambium, cortex, medullary rays, sclereids and parenchyma tissues. Periderm was multi layered composed of cork and phelloderm. Cork is exfoliative in nature and made up of rectangular cells with thickened walls. Groups of sclereids are present in the cork region. Vascular cambium cuts off primary phloem towards an outer surface and secondary phloem towards inner regions. Medullary rays are uni to biseriate. Abundant groups of sclereids are present in between the phloem parenchyma. Fibers are clearly seen in the radial longitudinal section (fig. 3) and Tangential longitudinal sections (fig. 4).

Fig. 1: Morphology of Bark

Powder microscopy

Powder microscopy showed the presence of uni to biseriate medullary rays, fibers with associated parenchyma, sclereids, parenchyma and pitted vessels (fig. 4). Fibers, sclereids and pitted vessels were stained pink with phloroglucinol and hydrochloric acid due to the presence of lignin in them. Presence of fibers is responsible for the observed striated or granular fracture.

Physiochemical study

The ash values usually represent the inorganic residue present in herbal drugs. The ash values are important indices to illustrate the quality as well as purity of herbal medicine. Physiochemical parameters were evaluated and the results were represented in table 1. Fluorescence study is an essential parameter for first line standardization of the crude drug. The powder exhibited brown, green and yellow colours.

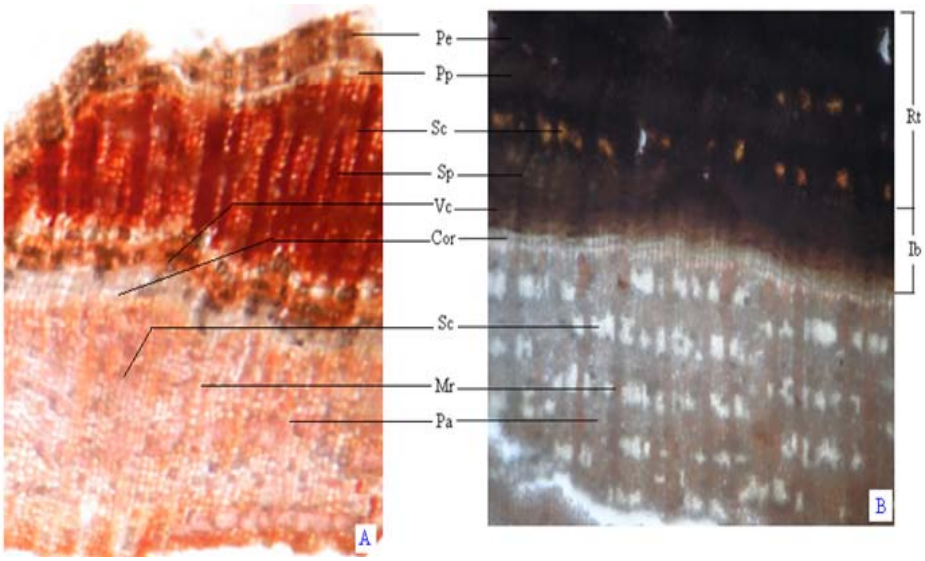

Fig. 2: Transverse section of bark. A- Stained section, B- Unstained section
Pe- periderm, Pp- Primary phloem, Sc- sclereids, Sp- secondary phloem, VcVascular cambium, Cor- cortex, Mr- Medullary ray, Pa- Parenchyma, Rt- Rytidoma/outer bark, Ib- inner bark when treated with different reagents indicating the presence of various phytoconstituents

Phytochemical screening

Most of the pharmcological activities possessed by the crude drugs is attributed to the presence of active principles. Among these active principles flavonoids and triterpenoids play a mojor role. Phytochemical investigation of methanolic extract of bark showed presence of alkaloids, flavonoids, steroids, triterpenoids and carbohydrates. The results of phytochemical screening were summarized in Table 3.

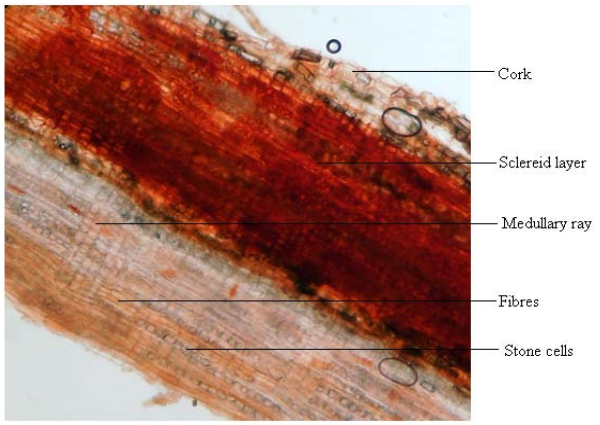

Fig. 3: Radial longitudinal section of Bark

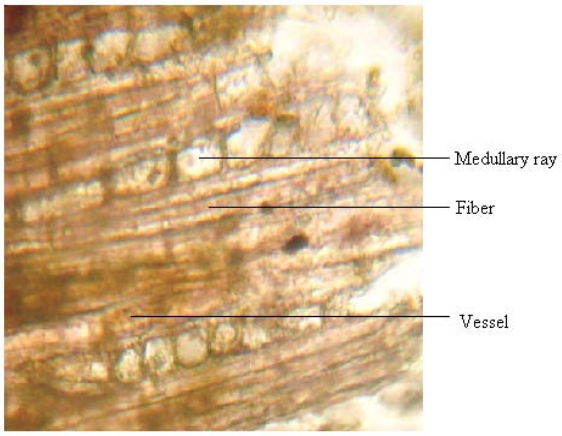

Fig. 4: Tangential longitudinal section of Bark

Table 1: Summary of Physicochemical analysis

S. No.	Physicochemical constants	% w/w
1	Total ash	7.5
2	Acid insoluble ash	17
3	Water soluble ash	2
4	Foreign organic matter	1.5
5	Moisture content not more than	1
6	Extractive Value (Methanol)	12

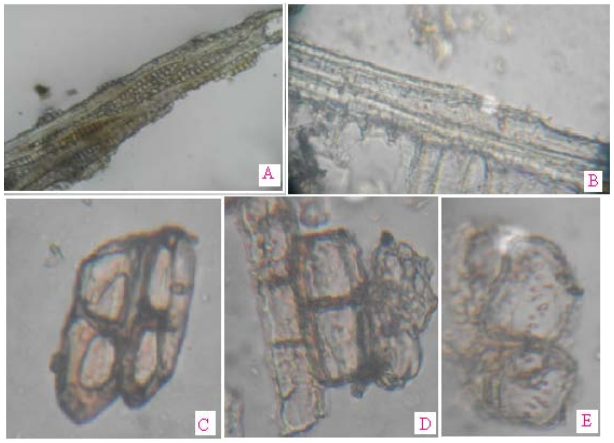

Fig. 5: Powder microscopy of bark
A- Medullary rays, B-Fibers with associated parenchyma, C-Sclereids, D- parenchyma, E- pitted vessels

DISCUSSION

Bark is the outermost layer of stems and roots of woody plants. Bark is useful to humans as well as to trees. Bark protects a tree. Bark from woody shrubs and trees were used for carbohydrate food, medicine, fiber and structural material [27]. Various products derived from bark were used by people as wall coverings, spices, flavorings, tannin, resin, latex, medicines, poisons and hallucinatory chemicals. Bark has been used to make cloth, canoes, and ropes and used as a surface for paintings and map making [28]. Tree bark is very complex in structure and has the potential of containing many primary and secondary metabolites. Products stored in the bark are useful for preparation of many drugs. The complex structure of the bark can be utilized for botanical identification to maintain the quality and purity of the drug [29].

Table 2: Summary of Fluorescence studies

Reagent	Normal light	Under UV Light	
		254 nm	365 nm
Powder as such	Light brown	Brown	Brown
Powder + Conc. HCl	Light brown	Light brown	Light brown
Powder + dil. HCl	Light brown	Light green	Light green
Powder + Conc. H_2SO_4	Dark brown	Dark brown	Dark brown
Powder + dil. H_2SO_4	Light brown	Light brown	Light brown
Powder + Conc. HNO_3	yellow	Greenish yellow	Greenish yellow
Powder + dil. HNO_3	Light brown	Light brown	Light brown
Powder + Iodine	yellow	Light yellow	Light yellow
Powder + $FeCl_3$	Greenish yellow	Green	Green
Powder + dil. NH_3	Light brown	Light brown	Light brown
Powder + Bromine water	Brown	Brown	Brown
Powder + NaOH	Light brown	Light brown	Light brown

UV- Ultra violet, Conc- Concentrated, dil- dilute, HCl- Hydrochloric acid, H_2SO_4- sulphuric acid, HNO_3- nitric acid, $FeCl_3$- ferric chloride, NaOH- sodium hydroxide, NH_3 – Ammonia

The present study was selected on the bark of *Callistemon citrinus* L. as the bark of trees possesses many uses as stated and there were no previous reports on the pharmacogenetics standards of the bark. Morphoanatomical features will help in the identification of the

crude drug and the powder microscopical characters will assist to detect the adulteration. Extractive values are primarily useful for the determination of exhausted and adulterated drugs. Extractive values are also useful to evaluate the chemical constituents present in the crude drug and help in estimation of specific constituents soluble in particular solvents[30, 31]. Ash values of the drug give an idea of earthy matter or the inorganic composition and other impurities presents along with the drug. The evaluated physicochemical parameters will be helpful in assessing the quality of the raw material.

Table 3: Summary of Phytochemical analysis

	Phytoconstituent	Methanol Extract
1	Carbohydrates	+
2	Proteins	-
3	Lipids	-
4	Alkaloids	+
5	Glycosides	+
6	Flavonoids	+
7	Tannins	+
8	Saponins	+
9	Steroids/triterpenoids	+
10	Coumarins	+
11	Phenolic compounds	+

+ Present, - Absent.

The pharmacological action of the crude drug is largely dependent on the metabolites present in it. Preliminary phytochemical screening of *Callistemon citrinus* L. bark revealed the presence many active principles like steroids, terpenoids and flavonoids which are responsible for most of the pharmacological activities. These simple and reliable pharmacognostic standards will help the manufactures for identification and selection of the raw material for the production of drugs.

CONCLUSION

As there were no reports on pharmacogenetics parameters of the bark of *Callistemon citrinus* L. the results will help in the quality control and standardization of the crude drug material. Further research can be carried out to isolate the active principles and evaluate them for the pharmacological efficacy of the crude drug. The reported pharmacognostic parameters can be considered as distinctive enough for authentification of this drug in herbal industry and can be included as microscopic standards in Indian herbal pharmacopoeia.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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