Research Article

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PHARMACOGNOSTICAL AND PHYTOCHEMICAL STUDY ON LEAF OF *COMBRETUM INDICUM* (L.)

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ABSTRACT

Combretum indicum (L.) is an extra-pharmacopeial drug with an abundance of medicinal properties. Throughout the world, different Parts of *Combretum indicum* (L.) is used in curing many ailments by folklore healers. In India, the drug *Combretum indicum* (L.) is commonly known by the name Madhu-Malathi and used by the folklore healers of different areas. Especially the use of *Combretum indicum* (L.) is seen among the folklore Practitioners of Dakshina Kannada District in curing ailments including paediatric conditions. Thus, it is very important to explore the identity of this drug by the proper Pharmacognostical and Phytochemical analysis. Hence, the present study was aimed at Pharmacognostical and Phytochemical study on Leaf of *Combretum indicum* (L.). The transverse section of the midrib of the leaf shows the presence of the endodermal layer; which is single-layered, surrounds by a vascular bundle and packed with starch grains. Endodermis covers the vascular bundle and contains several starch grains. The powder microscopy shows the presence of glandular trichome, calcium oxalate crystals etc. The percentage of extractive value was maximum in methanol which was 7.18%. Preliminary phytochemical study showed the presence of Proteins, Carbohydrates, Tannins, Steroids, Alkaloids, Triterpenoids, Starch, Resin and Phenols. The total ash attained was 7.84% and the water-soluble ash was 3.4%.

Keywords: *Combretum indicum* (L.), Pharmacognosy, Madhu-Malathi, Phytochemical study.

INTRODUCTION

The Combretaceae family often called the white mangrove family, are a family of flowering plants and includes 18 genera and 500 species. A large number of species of the Combretaceae family belong to mainly genera Terminalia and genera Combretum.¹ Habit: Mainly trees, shrubs or subscandent.^{2,3,4} The Drug *Combretum indicum* (L.) is commonly cultivated in gardens, found in thickets or secondary forests. In India, it is mainly found in the southern part of Karnataka, Tamil Nadu, Kerala and Hyderabad. This plant is a large straggling or climbing shrub that climbs through hard hook-like petioles of fallen leaves. Brown hairs give the younger parts a rusty appearance. Leaves are 9- 13×4.5-5 cm, elliptic entire, upper opposite and lower alternate, subcordate base, acuminate at apex. Flowers in terminal spikes white changing into red, reddish-purple or orange, exhibiting the range of colours in clusters on the same flower stalk. Bracts are ovate. Calyx: Tube 5-7 cm long, lobes triangular, acute. Petals 5, white changing to pink-red, ovate-oblong, alternating with calyx teeth, epispalous. Stamen 10, epispalous. The ovary is ovate to oblong, ovules 4, style filiform, exerted. Fruit is narrowly ellipsoid, 2.5 to 3 centimetres long, with five, sharp, longitudinal angles or wings. Seeds are pentagonal and black.^{4,5}

Even though reference about *Combretum indicum* (L.) in Ayurvedic classics is not explored properly, the wide range of availability as well the successful treatments done by the folklore healers throughout the Globe necessitate the exploration of its identity through the Pharmacognostical and other related studies.

Phytochemical screening yields major classes of constituents: alkaloids, carbohydrates, protein, amino acid, saponins, glycosides, steroids, tannins, flavonoids and phenolic compounds. Folklore people are using the different parts of this drug for curing many conditions. Among that the most useful part is the leaf. Leaves are applied to the head to relieve headaches. Pounded leaves externally for skin diseases. Decoction of boiled leaves used for dysuria. Ifugao migrants use it for headaches. Ayta communities in Dinalupan, Bataan, apply heated leaves on snake and animal bites.⁶ In India and Ambonia, leaves are used in a compound decoction to relieve flatulent distention of the abdomen. Leaves and fruits are reported to be anthelmintic; also used for nephritis. In Amboina, the compound decoction of leaves is used for flatulent abdominal distention. In Bangladesh, used for diarrhoea, fever, boils, ulcers and helminthiasis. Malays use leaf juice as a lotion for boils and ulcers; leaves are applied directly to headaches.⁷

The literary survey revealed that many studies have been done on the drug *Combretum indicum* (L.) but the information on Pharmacognosy of abundantly used part i.e. Leaf is minimal. Therefore, the present study is focused on the structural features of the leaf of *Combretum indicum* (L.) and preliminary phytochemical screening, pH analysis and TLC fingerprinting etc. of the authenticated leaf sample for standardization of the crude drug.

MATERIALS AND METHODS

Collection of samples: The leaves were collected from Moodbidri village of Dakshina Kannada district, Karnataka and

was authenticated by Subrahmanya Padyana, Director, ATMA Research Centre, Moodbidri, Karnataka, India. A herbarium No. ATMA/AC/2020/05 of this plant was deposited in the Alva's Traditional Medicine Archive & Research Centre Vidyagiri, Moodbidri. The leaf material was cleaned, dried in shade, powdered and stored in an air-tight container for further study.

Pharmacognostical study

Macroscopic evaluation- Done for the confirmation of its identity, determination of quality and detection of adulteration was done by a visual appearance by the naked eyes. Photo documentation of the macroscopic characters was done. Other sensory characteristics like odour, taste, and feel of the drug to the touch were also observed.

Microscopic study - The freshly collected *Combretum indicum* (L.) Leaf was cut into a thin transverse section with a sharp blade and the sections were stained with safranin. Transverse sections were photographed using Zeiss AXIO trinocular microscope attached with a Zeiss Axio Cam camera under bright field light. Magnifications of the figures are indicated by the scale bars.

Powder microscopy- Fine powder of *Combretum indicum* (L.) Leaf was mounted in Glycerine as well as with phloroglucinol and con. HCl. Gently heat the mixture and was observed under Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light.

Physicochemical study

Physical Standards: The air-dried leaf of *Combretum indicum* (L.) were powdered finely and subjected to various analyses such as Determination of moisture content, Determination of total ash, Determination of acid-insoluble ash, Determination of water-soluble ash, water-soluble extractive value, ethyl alcohol soluble extractive value, methanol soluble extractive value, chloroform soluble extractive value, Acetone soluble extractive value, Petroleum ether soluble extractive value and ash analysis.⁸

Preliminary phytochemical study: Various extracts were subjected to preliminary phytochemical studies to know the presence of Proteins, Carbohydrates, Tannins, Saponins, Flavonoids, Steroids, Alkaloids, Triterpenoids, Starch, Resins, Phenolics, Ellagic acid.⁹ The ash of the crude drugs were subjected to various chemical tests to find the inorganic components such as Carbonates, Fluorides, Chlorides, Sulphates, Chromate, Phosphate, Potassium, Sodium, Aluminium and Calcium.

TLC fingerprint profile: For TLC fingerprinting, methanol extract was prepared by extracting 2g of the powdered drug with methanol (3 × 10 ml) at room temperature, the extracts were combined, filtered and evaporated on a water bath. 10 mg of this dried extract was re-dissolved in 1 ml of methanol and 15µl of it was implicated with Camag Linomet IV applicator on a pre-coated silica gel G 60 F254 TLC plate (Merck) of uniform thickness of 0.2 mm. The plate was developed in solvent system Chloroform: Methanol: Formic acid (8.8:0.5:0.2) and visualized under UV 254 nm, UV 366 nm and 550nm. The fingerprint profiles were documented with the help of CAMAG Switzerland photo documentation unit Reprostar 3.¹⁰

PH value: The measure of pH is generally done with a suitable potentiometer known as the pH meter fitted with two electrodes, one constructed of glass and sensitive to hydrogenation activity and the other a Calomel reference electrode. The determination is carried out at 25 °C.¹¹

RESULTS

Pharmacognostical study

Macroscopic feature of the fresh leaf of *Combretum indicum* (L.): The leaf was observed to be elliptic in shape, Entire margin, 9- 13×4.5-5 cm, acuminate at apex, subcordate to rounded at base, reticulate venation, 8-10 pairs of veins are present membranous on touch opposite arrangement, bitter and has a specific odour. (Fig 1)

Microscopy of *Combretum indicum* (L.): Characters of the leaf-like upper epidermis, lower epidermis, parenchymatous cells, collenchyma cells, trichomes, xylem, and phloem. The whole of the midrib is filled with collenchyma with different types of trichomes such as covering and glandular. The midrib is almost triangular shows the presence of the endodermal layer; it is single-layered, surrounds by a vascular bundle, packed with starch grains. Endodermis covers the vascular bundle and contains several starch grains. Leaf surface (upper and lower surface) study shows the presence of epidermal cells, paracytic stomata, and subsidiary cells. (Fig 2)

The diagnostic character of powder & Powder microscopy: The powder was yellowish-green in colour, with a characteristic odour, bitter and Pungent as predominant taste, and also slightly sour. Under microscopic observation the powder shows the presence of vessels, covering trichome, glandular trichome calcium oxalate crystals, epidermal cells, paracytic stomata. The upper epidermis, lower epidermis, parenchymatous cells. Collenchyma cells, trichomes. The whole of the midrib is filled with collenchyma with a different types of trichomes such as covering and glandular. (Fig 3)

Physicochemical properties: Various Physicochemical constants like a loss on drying, total ash, acid insoluble ash, water-soluble ash, water-soluble extractive and alcohol soluble extractive were determined and are given in Table 1&2.

Preliminary phytochemical studies and Ash analysis: Ash analysis showed the presence of Potassium and the results of Preliminary phytochemical studies are given in Table 3.

TLC fingerprint profile: The photo documentation of the TLC fingerprint profile was viewed at 254 nm, 366 nm, and 550nm. (As Fig 4). HPTLC documentation of drug at 254nm, 366nm and 550 nm where 9, 12 and 15 peaks observed respectively.

Determination of PH- value: pH value of Leaf decoction of *Combretum indicum* (L) – 5.55

Fig 1: Macroscopy of Leaf of *Combretum indicum* (L.) A-Leaf , B- Phyllotaxy

Fig 2: Microscopy of *Combretum indicum* (L.) leaf; A-T.S of Petiole, B-T.S of Midrib

Fig 3: Powder microscopy of the leaf of *Combretum indicum* (L.).A-Fragments of fibre, B-Trichome, C- Fragments of collenchyma, D-Rosette crystal, E-Cells with rosette crystals, F-Fragments of fibre

Fig 4: TLC Fingerprint of *Combretum indicum* (L.) leaf

Table 1: Physical standards

Parameter	Result
Total ash	7.84%
Water-soluble ash	3.4%
Acid insoluble ash	1.2%
Percentage of moisture content	7.8%

Table 2: Percentage of extractive values

Solvents	Percentage of extract of <i>Combretum indicum</i> (L.)
Water	5.28%
Ethanol	2.44%
Methanol	7.18%
Chloroform	3.56%
Petroleum Ether	1.5%
Acetone	3.48%

Table 3: Phytochemical components present in *Combretum indicum* (L.)

Components	<i>Combretum indicum</i> (L.)
Proteins	Present
Carbohydrates	Present
Tannins	Present
Saponins	Absent
Flavonoids	Absent
Steroids	Present
Alkaloids	Present
Triterpenoids	Present
Starch	Present
Resin	Present
Ellagic acid	Absent
Phenols	Present

DISCUSSION

The previous analytical studies done on the leaves of *Combretum indicum* (L.) showed that Leaves yield rutin, trigonelline, L-proline, L-asparagine, and quisqualic acid. Petroleum ether extract of leaves yielded palmitic acid (27.73%) as the major component of the saponifiable component, and α -amyrin, of the unsaponifiable portion. Crude protein was 2.06%. An unusual protein, dihydro-quisqualic acid, was isolated for the first time.¹²

Studies have proven of *Combretum indicum* (L.) is having Pharmacological activities such as; dose-dependently inhibited acetylcholinesterase activity.¹³ Larvicidal activity¹⁴, antipyretic activity¹⁵, Anti-Inflammatory activity¹⁶ immunomodulatory activity¹⁷, Anticonvulsant Activity¹⁸, Anthelmintic¹⁹, Antihyperlipidemic²⁰, Anti microbial²¹, Anti Mutagenic²², Antibacterial^{23,24}, Antioxidant activity²⁵ etc.

The main identification feature of the drug *Combretum indicum* (L.) observed during the study is the climbing nature of the plant where the plant climbs through the hook-like petioles of the fallen leaves. The fresh leaves were having a peculiar smell which can also be taken as a key identification feature. The leaves are leathery in touch and the arrangement of the leaves on the upper side of the petiole looks like having opposite arrangement whereas the lower side appears to be an alternative. The tender leaves are having brownish-red colour. The dried leaves are greenish-yellow in colour with a specific odour.

Microscopic analysis showed the presence of epidermal cells, paracytic stomata, and subsidiary cells in the upper and lower Leaf surface. The whole of the midrib is filled with collenchyma with different types of trichomes such as covering and glandular. Endodermis covers the vascular bundle which is packed with starch grains. HPTLC documentation of drug at 254nm, 366nm and 550 nm where 9, 12 and 15 peaks observed respectively. pH value was - 5.55 which indicates the alkaline nature of the drug.

CONCLUSION

Nowadays the people irrespective of the region are in search of a herbal cure to avoid the obnoxious effects of the commonly

available treatment modalities. *Combretum indicum* (L.) is one of the unexplored plants with various pharmacological activities. It is very important to analyse the Pharmacognosy of each part of this plant to avoid the confusions which might creep in the process of identification. The present study analysed the Pharmacognosy and the related parameters which helps in the proper identification of the leaf of the *Combretum indicum* (L.) both macroscopically as well as microscopically along with the physical parameters, ash analysis, PH determination, and preliminary phytochemical analysis and also through the fingerprint profile.

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