Pharmacognostic study of root of *Combretum albidum* G. Don

Ashish S. Zalke*, B. Duraiswamy and Upendra B. Gandagule

Department of Pharmacognosy, JSS College of Pharmacy, Rocklands, Ootacamund-643001

**ABSTRACT**

**Background:** *Combretum albidum* G. Don commonly known as Buffalo calf. The *C. albidum* is used for diverse health ailments in traditional and folklore remedies. **Objective:** The present study was undertaken to lay down pharmacognostic and phytochemical standards for *Combretum albidum* G. Don. **Material and Methods:** The pharmacognostic studies were carried out in terms of macroscopic, microscopic, physicochemical, fluorescence and phytochemical analysis. Physicochemical parameters such as total ash, moisture content, extractive values are determined as per WHO guidelines. The microscopical features of root components are observed with Nikon labphoto 2 microscopic unit. **Results:** The morphologically, root are pale brown colored, cylindrical with slightly bitter in taste and agreeable odour. Distinguishingly roots showed root scares, rootlet and fibrous fracture. Microscopy of root consists of thick epidermis, periderm, and cortex with sclerenchyma cells. Vascular cylinder includes thin phloem and thick, solid and dense xylem cylinder. The prismatic calcium oxalates were observed. Powder microscopy of root revealed that fibres were fairly wide and densely packed with starch grains. The fluorescence and physicochemical standards for root were established. Phytochemically root showed the presence of carbohydrate, glycoside, saponin, flavonoid, phytosterols and phenolic compounds. **Conclusion:** There is no pharmacognostic and phytochemical reports on *C. albidum* to authenticate and differentiate them from similar species. Therefore, present work was undertaken and established the pharmacognostic and phytochemical characteristics of *C. albidum* and diagnostic features to differentiate it. **Keywords:** *Combretum albidum*, Microscopy, Macroscopy, Phytochemical, Fluorescence analysis.

**INTRODUCTION**

Natural products have served as the source and inspiration for a large fraction of the current pharmacopoeia. Although estimates vary depending on the definition of what is considered a natural product-derived drug, it is safe to say that between 25% and 50% of currently marketed drugs owe their origins to natural products.[1] *Combretum albidum* (*C. albidum*) G. Don belonging to the family Combretaceae and commonly known as ‘Buffalo calf’ in English (Figure 1A). It is extensive woody twine occupying the canopy of host tree. Its distribution is restricted to the semi evergreen and dry deciduous forest, along the river bank.[2] Puliyan tribes in Sirumalai hills of Eastern Ghats used fruit for the treatment of diarrhoea and dysentery. [3] Furthermore, tribal of Kalrayan and Shervarayan hills’s uses wiry-stem, seed oil and root for the treatment of eye problems, eczema and antimalarial.[8] Traditionally, the stem barks used in treatment of jaundice,[10] skin disease,[11,12] and leaf for peptic ulcer.[13] Mahida and Mohan reported antibacterial activity of the plant.[14] Despite of wide traditional and tribal use of *C. albidum*, the plant is not pharmacognostically standardized. Hence, the present investigation was under taken with the objective of evaluating various parameters such as macroscopic, microscopic characters and phytochemical evaluation of the plant.

**MATERIAL AND METHODS**

**Procurement of plant materials**

Fresh roots of *C. albidum* were collected from forest of Tirupati, Andhra Pradesh (India) in the month of
September, identification and authentication (SVU/SC/19/82/10–11) of the plant was done by Dr. Madhava Chetty, Professor, Department of Botany, Sri Venkateswara University, Tirupati.

Macroscopic evaluation

The root was separated from other parts, washed, cleaned, and dried for further use. The macroscopic characters of the fresh root were noted: color, odour, taste, size and shape, touch, texture and fracture.[10,11]

Physicochemical and phytochemical analysis

The powdered root was analyzed for physicochemical properties such as ash values and extractive values using official method.[12,13] Furthermore, preliminary phytochemical screening was carried using qualitative tests.[14] Fluorescence analysis

Fluorescence analysis of root powder was carried out under visible light and short UV light after treatment with various acids, alkalis, other reagents.[15,16] Microscopic evaluation

In this study, transverse sections of root were studied under photomicrograph. The various identifying characters were studied with or without staining and recorded. The normal healthy root were cut and removed from the plant and fixed in FAA (Formalin 5ml+acetic acid 5ml+70% ethyl alcohol 90ml). After 24hrs of fixing, the specimens of roots were dehydrated with graded series of tertiary-Butyl alcohol as per the schedule given by Sass, 1940.[17] Infiltration of the specimens roots was carried by gradual addition of paraffin wax (mp 58–60°C) until tertiary Butyl alcohol solution attained super saturation. The specimens of roots were cast into paraffin blocks. The paraffin embedded specimens of roots were sectioned with the help of rotary microtome. The thickness of the sections was 10–12µm. Dewaxing of the sections was by customary procedure.[18] The sections were stained with toluidine blue as per the method published by O’Brien et al.[19] Since toluidine blue is a polychromatic stain. The staining results were remarkably good and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. Wherever necessary sections of root were also stained with safranin and fast green and iodine potassium iodate (for starch).

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon labphoto 2 microscopic unit. For the study of crystals, starch grains and lignified cells, polarised light was employed. Since these structures have birefringent property, under polarised light they appear bright against dark background. Magnifications of the figures are indicated by the scale bars. Descriptive terms of the anatomical features are as given in the standard anatomy books.[20,21]

Powder microscopy

The dried roots were powdered. Powdered material was cleared with sodium hydroxide and mounted in glycerine medium after staining. Different staining reagents such as toluidine blue, safranin, fast green and iodine were used. Different cell component were studied and measured using photomicrography.
RESULTS

Macroscopic evaluation

Externally the root had shown the pale brown color with distinct color difference in the root bark. The outer surface of the root bark was pale brown with dark reddish brown internally. The root is cylindrical in shape with size of 5–10 × 2.5 to 5 cm [l × d] slightly bitter in taste and agreeable odour. The root shows root scares and presence of rootlet on surface. The cortex was slightly yellowish in colour with fibrous fracture (Figure 1B).

Physicochemical and phytochemical analysis

Physicochemical analysis of root powder viz. ash values, extractive values and moisture content were presented in table 1. The preliminary phytochemical analysis of ethanolic extract of root was shown the presence of glycoside, carbohydrate, flavonoid, phytosterols, phenolic compounds and saponin.

Fluorescence analysis

The fluorescence analysis of C. albidum root under day and UV (short 254nm) light was recorded in table 2.

<table>
<thead>
<tr>
<th>Physico-chemical parameters</th>
<th>Root powder (%w/w)</th>
</tr>
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<tbody>
<tr>
<td>Total ash</td>
<td>6.40 ± 0.08</td>
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<tr>
<td>Water soluble ash</td>
<td>5.750 ± 0.08</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>0.408 ± 0.32</td>
</tr>
<tr>
<td>Water soluble</td>
<td>4.52 ± 1.74</td>
</tr>
<tr>
<td>Alcohol soluble</td>
<td>7.59 ± 0.09</td>
</tr>
<tr>
<td>Moisture content</td>
<td>10.27 ± 0.11</td>
</tr>
</tbody>
</table>

Table 2. Fluorescence analysis of root powder of C. albidum.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day light</th>
<th>UV light (Short, 254)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powder(P) as such</td>
<td>Buff</td>
<td>Grayish green</td>
</tr>
<tr>
<td>P + 1N NaOH</td>
<td>Dark reddish brown</td>
<td>Dark black</td>
</tr>
<tr>
<td>P + Picric acid</td>
<td>Creamsom red</td>
<td>Yellowish green</td>
</tr>
<tr>
<td>P + 1N HCl</td>
<td>Creamsom pink</td>
<td>Yellowish green</td>
</tr>
<tr>
<td>P + 1N HNO$_3$</td>
<td>Creamsom pink</td>
<td>Yellowish green</td>
</tr>
<tr>
<td>P + 5% Iodine</td>
<td>Creamsom Black</td>
<td>Green</td>
</tr>
<tr>
<td>P + 5% FeCl$_3$</td>
<td>Dark green</td>
<td>Black</td>
</tr>
<tr>
<td>P + HNO$_3$ + NH$_3$</td>
<td>Reddish</td>
<td>Yellowish green</td>
</tr>
<tr>
<td>P + 1N NaOH in methanol</td>
<td>Brown</td>
<td>Black</td>
</tr>
<tr>
<td>P + Methanol</td>
<td>Slightly yellow green</td>
<td>Yellowish green</td>
</tr>
<tr>
<td>P + 50% HNO$_3$</td>
<td>Dark brown</td>
<td>Dark green</td>
</tr>
</tbody>
</table>

Microscopic evaluation

Roots of different thickness were studied. A thin root measuring 750μm in diameter consists of a thin epidermal layer and inner initial stage of phellem development. The cortex consists of outer zone of two or three layers of small compact parenchyma cells and inner wide, radially oblong air chambers separated from each other by thin partitions filaments (Figure 2A). The central part was occupied by a circular vascular cylinder of 400μm thickness. The vascular cylinder was composed of a thin endodermoid layer, outer sclerenchymatous ground tissue and central core of gelatinous fibres. There were narrow, circular xylem elements which include metaxylem and exarct protoxylem elements (Figure 2B).

Thicker root of 1.1mm thick consists of thick darkly stained epidermis, narrow superficial periderm and a few layers cortex with thick discontinuous cylinders of sclerenchyma cells (Figure 3A). The vascular cylinder of the thick root includes a thin layer of phloem and thick, solid and dense xylem cylinder (Figure 3A). The xylem cylinder includes dense ground tissue of gelatinous fibres, thin straight xylem rays and wide circular thick walled vessels which were solitary and diffuse (Figure 3B). The vessels were surrounded by thin sheath of fibres all around.
Phloem surrounds the xylem cylinder in uniform continuous layer of radial rows of cells (Figure 4B). Calcium oxalate crystals were common in the phloem parenchyma and adjacent to phloem sclerenchyma cylinders (Figure 5). The crystals were mostly prismatic type and they range from rhomboidal to cuboidal shape.

**Powder microscopy**

The root-powder shown isolated, densely crowded xylem elements. The elements include: The vessel elements were wide, long cylindrical cells (Figure 6A). Their ends were either blunt (Figure 6B) or they had short tails (Figure 6A). At the ends of the vessel elements, simple, circular, oblique or horizontal perforations were seen (Figure 6B, 6E and 6F). On the lateral walls occur dense pits. The pits were 6 or 6 seriate in narrow vessel (Figure 6E) multiseriate in wider vessel (Figure 6F). The vessel elements were 250–450µm long.

Xylem fibres were abundant in the powder (Figure 6A). They were long, narrow with tapering ends (Figure 6C). The walls were thick and the lumen was narrow. Some of the fibres were fairly wide and densely packed with cylindrical starch grains (Figure 6D). These fibres called storage fibres. The fibres were 800µm to 1mm long and 15–20µm wide.

**DISCUSSION AND CONCLUSION**

The comprehensive analysis of the pharmacognostic features of root of *C. albidum* here presented for the first time may help to evaluate the usefulness of these characters in establishing the botanical identity of the plant. According to Metcalfe, any exercise that involves the identification of vegetable material when it is in a fragmentary or partly decomposed condition can be achieved only by the methods of comparative histology. This pointing in establishing the identification of economic plant products ranging
from timbers to foodstuffs as well as crude drugs of vegetable origin. Adulterants and substituents can also be detected.

The microscopy of *C. albidum* showed all general characteristics of bark with some distinct differentiation. In the microscopic studies of the young root indicated a thin epidermal layer and inner initial stage of phellem development and in thicker root, thick darkly strained epidermis, narrow superficial periderm and a few layers cortex with thick discontinuous cylinders of sclerenchyma cells. Calcium oxalate crystals were common in phloem parenchyma and phloem sclerenchyma. They are mostly prismatic type and range from rhomboidal to cuboidal shape. These are definitely useful for identification of plant microscopically. In the powder microscopy of root, the fibres were densely packed with cylindrical starch grains. Percentage extractives and ash analysis were carried out. Results showed that water soluble ash of root is more than acid insoluble ash. Alcohol soluble extractive value had shown two times more than water soluble extractive value. Ash values of drug give an idea of earthy matter or the inorganic composition and other impurities present along with drug. 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 also help in estimation of specific constituents soluble in particular solvents. The fluorescent analysis under day light and UV light by treatment with different chemical showed different colour. Results of fluorescent analysis of the root showed buff colour for root powder as such in day light, grayish green colour for root powder as such in UV light. Dark reddish brown colour for root powder mounted in 1N NaOH as such in day light while dark black colour for root powder as such in UV light. Creamsom pink colour for root powder mounted in 1N HCl as such in day while yellowish green colour for root powder as such in UV light. This analysis suggests that root extract of *C. albidum* probably contain active chemical constituent(s) and this provide the basis for their folkloric use.
use as a cure for some human ailments. In conclusion, the parameters which are reported for the first time could be useful for identity and authenticity of this medicinal plant.

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**REFERENCES**


