ABSTRACT

Aim & Background: Resin of Canarium strictum Roxb., is an imperative commodity in traditional medicine in South and South East Asia. The current study aims to establish the quality control parameters for the bark as it secreted more useful resin. Methods: Anatomical studies and physiochemical evaluation of the bark was carried out according to the standard procedure was given in WHO/QCMMP guidelines and Indian Ayurvedic Pharmacopoeia. The anatomical studies of tissues were taken as photographs with different magnifications by using Nikon lab photo 2 microscopic Unit. The elemental analysis was done by using Perkin Elmer 5000 an atomic absorption spectrophotometer. Results: The different cell components were studied and measured quantitatively. The calcium oxalate prismatic crystals were estimated about 10×10 or 10×5µm in size. The sclereids were very long of unlimited length and 10µm in thickness. The long narrow lignified fibers has been found and estimated about 210–260µm long and about 10µm thick. The height of the ray is up to 350µm in height and 60µm in breadth. The physiochemical parameters such as total ash and acid insoluble ash (5.52% w/w, 2.66% w/w, respectively), extractive values (aqueous 4.55% w/w and alcoholic 6.05% w/w), foreign organic matter (2.4%) and loss on drying (7.09% w/w) were also estimated. An elemental analysis result shows the quantity of elements (µg/g) were present in the bark powder. Among the elements Mn-73.6, Cu-65.4, Cr-49.5 were major contents, while Pd-25.6 and Zn-35.4 were the minor contents. Conclusion: The current study report will be unique finger print for microscopical evaluation of bark of this tree and also used to differentiate the plant species among Canarium L.

Keywords: Burseraceae, Western Ghats, Quality control, Siddha medicine, Black dammer, Rheumatism.

INTRODUCTION

Canarium L. belongs to the family of Burseraceae Kunth., in the order Sapindales Juss. ex Bercht. & J. Pearl. This family comprises of 18 genera and estimated about 700 species of tropical trees.[1] Canarium strictum species producing resin, is a rich source for making fragrance smoke (Sambrani or Black dammer) and it is given for the treatment of bronchial diseases and orally given resin powder used to cure rheumatism and it is one of the major drug in Siddha medicine.[2] Black dammer resin is also used as an alternate for burgundy pitch in making medical plasters.[2,3] Extreme usage of resin in industry as well as in traditional medicine, the species currently positioned on the IUCN red list as endangered species in the regions of Tamilnadu, Kerala and Karnataka state in South India.[3]

Canarium strictum Roxb. Syn C. sikkimense King (Burseraceae) yields the resin, which is harvested from evergreen forest Nilgiri Biosphere Reserve (NBR) and throughout Western Ghats of Kerala, a biodiversity centre.[4] Canarium sikkimense King is called as gogul dhuup in Nepal,[5] Black dammar is being used traditionally to treat rheumatism, asthma, coughs, fever, epilepsy, chronic skin diseases and hemorrhage.[6]

The black dammer resin which is collected from wounded trunk of the tree contains triterpenoids such as α-amyrin, β-amyrin, β-amyrin acetate, (+) junenol, canarone, epi-khusinol and -Ψ-taraxasterol and epi-Ψ-taraxastane diol.[7] Resin and its isolated compounds exhibited anti
inflammatory, analgesic and anti-bacterial and anti-fungal activities.[8,9]

Unlike many other Canarium species; the fruits of C. strictum are not edible. The harvest of black dammar is permitted for trade in Kerala, but in Tamil Nadu, as a conservation measure, harvest is permitted only for personal or home use. The resin is important worship item for tribal groups of nilgiris district, particularly baduga they hold 3 to 4kg in home and they collect resin only from female tree rather than male.[4]

No studies were carried out on the synthesis of black dammar, synthesis of most of the constituents of resin in Canarium species, is considered to be produced by making incision over the bark.[10] As an alternative of selecting other part of this plant for setting quality control parameters, choosing stem bark was found to be significant as it is secreted more useful resin. As a result the current study has decided to investigate the anatomical, physicochemical characters and presence of elements in much used bark of the C. strictum.

MATERIALS AND METHODS

Collection of specimens

The fresh bark was collected early in the morning during the rainy season in July 2011 from the tall tree of Canarium strictum in Rayairath garden, Pattikadu, Thrissur district, Kerala. The plant material was taxonomically identified by Prof. P. Jayaraman, Taxonomist, Plant Anatomy Research Centre, Chennai. The voucher specimen (No.PARC/2010/1475) was deposited in medicinal plant documentation unit in pharmacognosy and phyto chemistry department, Nehru College of Pharmacy, Pampady, Thrivulvamala-680597, Thrissur district, Kerala state, India as future reference. For anatomical studies, the collected bark was fixed in FAA (Formalin-5ml+ Acetic acid-5ml + 70% Alcohol-90ml). After 24 hrs of fixing, the specimen was dehydrated with graded series of tertiary–Butyl alcohol (TBA).[11] Infiltration of the specimen was carried by gradual addition of paraffin wax (melting point 58–60 C) until TBA solution attained super saturation.[12] The specimen was cast into paraffin blocks.

Sectioning

The paraffin embedded specimen was sectioned with the help of Rotary Microtome. The thickness of the sections was 10–12μm. De waxing of the sections was done by standard procedure.[13] The sections were stained with toluidine blue,[4] as it 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 were also stained with safranin and fast-green and iodine (for Starch). Glycerine mounted temporary preparations were made for cleared materials. Powdered materials of different parts were cleared with sodium hydroxide solution and mounted in glycerine medium after staining.[11] Different cell components were studied and measured quantitatively.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have fingerprint property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Anatomical features of description were as given from the standard anatomy book.[15,16]

Histochemical colour reactions

The micro-chemical tests for histological region were performed according to the standard methods.[13,17–19]

Behaviour of powder with different chemical reagents

The powder material was treated with different chemical reagents to detect the phytoconstituents with colour changes under ordinary daylight by the standard method.[20]

Colour and consistency of extracts

Colour and consistency of extracts were observed by standard method.[20]

Estimation of inorganic constituents

To calculate the inorganic metal content, 1g of the completely dried powder sample was digested with concentrated nitric acid and perchloric acid (3:1) until a clear solution was obtained. After cooling, the solution was made up to a specific volume with demineralized water and analyzed in an atomic absorption spectrophotometer (Perkin Elmer 5000).[21]
Fluorescence analysis of powder and extracts

The bark extracts were examined and analyzed in daylight, short and long UV light for fluorescence, according to the standard methods.[22]

Determination of physicochemical parameters

Loss on drying, Water soluble extractive value, Alcohol soluble extractive value, Foreign organic matter, Total ash value, Determine the pH of 1% crude drug solution. Crude fiber content of bark of Canarium strictum Roxb., were evaluated according to the standard procedures.[23–25]

Preliminary phytochemical screening evaluation was carried out by using standard procedure.[26–28]

RESULTS

Physicochemical evaluation report was depicted in table 1&2. The coarse dried bark powder was extracted with hexane, chloroform and ethyl acetate and ethanol by successive solvent hot percolation method. The percentage of yield of each extract was calculated and its color, consistency was noted. Elemental analysis was carried out by standard method. The percentages of water and alcohol soluble matter were determined and all these results were depicted in table 1.

Qualitative phytochemical analysis of successive solvent extracts was performed; the report shows the presence of triterpenoids in hexane, chloroform and ethanol extracts and flavanoids phenolic compounds and tannins and saponins present in ethyl acetate and ethanol extracts. Almost all the successive solvent extracts exhibited yellowish green colour fluorescence both in short as well as long UV. The dried bark powder shows the pale yellow colour in day light. Histochemical colour reaction of the bark powder of Canarium strictum shows the presence of lignin and tannins and calcium oxalate. The starch and proteins were absent.

In fluorescence analysis, both in long and short UV, pale yellow colour was exhibited by hexane, chloroform and ethanol extracts but in contrast ethanol extract shows the yellowish black in short UV and blackish brown in long UV. The aqueous extract shows the pale yellow colour in short UV and yellowish brown in long UV.

<table>
<thead>
<tr>
<th>Table 1. Physicochemical values of bark of Canarium strictum Roxb.</th>
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<tbody>
<tr>
<td><strong>Parameters</strong></td>
</tr>
<tr>
<td>I. Organoleptic characteristics</td>
</tr>
<tr>
<td>Appearance</td>
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<tr>
<td>Colour</td>
</tr>
<tr>
<td>Odour</td>
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<tr>
<td>Taste</td>
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<tr>
<td>II. Loss on drying</td>
</tr>
<tr>
<td>III. pH values</td>
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<tr>
<td>pH of 1% aqueous solution</td>
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<tr>
<td>IV. Ash values</td>
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<tr>
<td>Total ash</td>
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<tr>
<td>Water soluble ash</td>
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<tr>
<td>Acid insoluble ash</td>
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<tr>
<td>Sulphated ash</td>
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<tr>
<td>V. Alcohol soluble matter</td>
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<tr>
<td>VI. Water soluble matter</td>
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<tr>
<td>VII. Successive extractives</td>
</tr>
<tr>
<td>Hexane extract</td>
</tr>
<tr>
<td>Chloroform extract</td>
</tr>
<tr>
<td>Ethanol extract</td>
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<tr>
<td>Aqueous extract</td>
</tr>
<tr>
<td>VIII Crude fiber content</td>
</tr>
<tr>
<td>IX. Foreign organic matter</td>
</tr>
<tr>
<td>X. Inorganic constituents present in dried bark of Canarium strictum</td>
</tr>
<tr>
<td>Zn</td>
</tr>
<tr>
<td>Mn</td>
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<tr>
<td>Cu</td>
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<td>Cr</td>
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<tr>
<td>Pd</td>
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</tbody>
</table>
N-Hexane, chloroform and ethyl acetate extracts were shows solid consistency whereas as alcohol extract was in semi solid consistency. Table 2 depicted the behavior of the bark powder with different chemical reagents.

Microscopical characters of transverse sections and powder microscopical characters of bark of *Canarium strictum* were studied and the findings were described here under with related photographs were shown in different panel of figures.

**Microscopical characters of *Canarium strictum* stem bark**

The surface of the bark is rough and irregularly fissured. The periderm peels off in to irregular pieces (Fig. 1) Inner to the periderm occur thick zone of collapsed phloem which includes thick tangential blocks of sclereids and long darkly stained thick tangential lines of collapsed phloem. Inner to the collapsed phloem zone is a narrow

<table>
<thead>
<tr>
<th>S.No</th>
<th>Treatment with Reagents</th>
<th>Under Ordinary light</th>
<th>Under UV light</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Long wavelength</td>
<td>Short wavelength</td>
</tr>
<tr>
<td>I</td>
<td>Powder as such</td>
<td>Pale yellow</td>
<td>Pale yellowish</td>
</tr>
<tr>
<td>II</td>
<td>Dry powder was placed on glass slide affixed with nitrocellulose</td>
<td>pale Yellow</td>
<td>Yellow</td>
</tr>
<tr>
<td>I</td>
<td>Powder treated with 1M NaOH in Methanol</td>
<td>Pale Reddish</td>
<td>Dull reddish brown</td>
</tr>
<tr>
<td>II</td>
<td>Powder treated with 1N NaOH in Methanol, dried and then mounted in Nitrocellulose in Amylacetate</td>
<td>Pale reddish</td>
<td>Reddish black</td>
</tr>
<tr>
<td>V</td>
<td>Powder treated with 1M HCl</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td>VI</td>
<td>Powder treated with 1M HCl, dried and then mounted in nitrocellulose in amylacetate</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td>VII</td>
<td>Powder treated with 1M NaOH in water</td>
<td>Pale Reddish</td>
<td>Dark Reddish</td>
</tr>
<tr>
<td>VIII</td>
<td>Powder treated with 1M NaOH in water, dried and then mounted in nitrocellulose in amylacetate</td>
<td>Reddish brown</td>
<td>Pale Reddish</td>
</tr>
<tr>
<td>IX</td>
<td>Powder treated with 50% HNO3</td>
<td>Yellow</td>
<td>Yellowish green</td>
</tr>
<tr>
<td>X</td>
<td>Powder treated with 50% H2SO4</td>
<td>No change</td>
<td>No change</td>
</tr>
</tbody>
</table>

**Figure 1.** T.S. outer bark showing flakes of periderm and collapsed phloem with sclereids and crushed tangential bands of sieve elements. Cph Collapsed phloem, NCPh Non collapsed phloem, Pe- Periderm, SC- Secretary cavity, Sel- Sclereids.
zone of non collapsed phloem. In this zone there are only limited numbers of small sclereid masses. The phloem cells are intact and fairly well preserved. (Fig. 1)

In the collapsed phloem the parenchyma cells are dilated, while the sieve elements are compressed into thick tangential lines. The sclereids are branchy sclereid type with thick lignified walls and dense simple pith.

Calcium oxalate crystals are fairly abundant in the collapsed phloem zone. The crystals are either prismatic type or druses. The crystals are mostly associated with the sclereids bands of the collapsed phloem.

In tangential longitudinal sectional view TLS the characters of the phloem rays were studied. The phloem rays are mostly biseriate or three seriate, short and spindle shaped. The rays are heterocellular (Fig. 2.1, 2 & 3). The ray consists of long conical upright cells and upper and lower ends.

The middle portion consists of circular or angular compact procumbent cells. The rays are not storied. The height of the ray is up to 350µm in height and 60µm in breath. The axial parenchyma cells are seen in vertical row of long cylindrical cells. The sclereids are also visible in TLS.

In Radial longitudinal section RLS view the rays appear in horizontal bands. The rays have upright cells on the upper and lower ends and horizontally oriented procumbent cells with wavy cell walls. The parenchyma cells are seen in vertical strands and the cells are vertically elongated and thin walled. Sclerenchyma elements are also seen in vertical bundles.

Calcium oxalate prismatic crystals are abundant in the collapsed phloem. They are mostly located within the lumen of sclerenchyma cells. (Fig. 3.1 & 2) The crystals are 10×10 or 10×5µm in size.

**Powder microscopy**

In the powder of the bark the following elements were observed.

**Fibre sclereids**

These are fiber like in length and thickness. However the cell walls are very thick and the cells lumen is very narrow. The sclereids are very long of unlimited length and 10µm in thickness.
Crystals

Prismatic crystals of calcium oxalate are seen associated with fibers of sclereids. The crystals are located within the lumen of the fibers or outside the fiber, located in the axial parenchyma cells. These crystals are either cuboidal or rectangular in shape. The crystals are also seen away from the cells and scattered in the medium. The free crystals are different type of prismatic crystals.

Sclereids

Branchy sclereids are abundant in the powder. They are cuboidal or rectangular; they have thick lignified cell walls and narrow lumen. Dense simple pits are seen on all walls of the sclereids.

Fibers

Long narrow lignified fibers are also seen commonly in the powder. The fibers are all narrow type. They have thick lignified walls. The fibers are all narrow type. They have thick lignified walls and pointed ends. Pits are not evident in the fibers. The fibers are 210–260μm long and about 10μm thick.

DISCUSSION

In the view of fact that resin is created by trees to defend against possible damage from abiotic or biotic stress. According to the type of species resin might be gathered in resin canals or resin pockets in the wood or the bark. In number of species peripheral rows of distressing resin canals are induced after wounding. Resin canals (axial or radial) are extended extracellular structures that allow long distance resin transport. Resin usually secreted through secretory cells known as ‘epithelium’ that surrounds resin canals or resin pockets.

Black dammer resin can be collected after making injury of outer bark shown that resin secretory structure may be in radial resin canals also near xylem and phloem and sclerenchyma part of the inner bark. Bark and wood of the tree found to be fibrous has 53.5% of crude fiber content.

During our Nilgiri Biosphere Reserve (NBR) visit we found that the dark brown colour resin was collected from the wounded trunk of the tree was given orally in the form of decoction, twice a day used to treat body pain and arthritis and the resin in oily preparation named as “Sivappukkul” is applied externally to treat skin inflammation. In eastern Arunachal Pradesh, Khamti tribal were applied melted resin over the skin to reduce the inflammation induced by contact poison of brown hairs of caterpillar.

Entire plant excluding root of Canarium strictum alcoholic extracts was evaluated biologically for various activities such as Antibacterial; Antifungal; Antiprotozoal; Anthelmintic; Antiviral; Anti-implantation in rats; Hypoglycemic; Effect on cardiovascular system; Effect on respiration; Effect on blood pressure; Effect on preganglionically stimulated nictititating membrane; Heart rate; Effect on acetycholine; Effect on adrenaline; Effect of histamine on guinea pig ileum; Effect on central nervous system and gross behavior. Gross effects, analgesia; Supramaximal electroshock seizure pattern test. Apart from root the whole plant was not exhibited any potent biological activity.

Literature shows that the genus Canarium L. contains terpenes which include monoterpenes, triterpenes and tetraterpenes like carotenoids, sesquiterpenes, cyclohexane and sterols and carboxylic acids, coumarins, furans, lipids and phenolic compounds such as flavonoids, tannins, phenolic acids. Our studies found through the qualitative analysis the presence of flavonoids, phenolic acids saponins, lignin and tannins and terpenoids and calcium oxalate in the bark.

The cheapest way to identify the plant and to develop monograph is to studies of anatomical characters including macro and microscopical characters are fundamental of Pharmacognostic evaluation of crude drugs.

CONCLUSION

The current study has been established the anatomical character and physicochemical characters and quantitative measurement of cell components and elemental analysis of the bark. The data we provided can be considered as finger print towards correct identification of Canarium strictum from other plant species of the same genus Canarium L. By using GCMS, the current study will be extended to investigate the presence of terpenes in the essential oil which is distilled from the resin of the tree. The major terpene of the essential oil will be isolated and screened for antinociceptive activity to prove folkloric report. Optimistically this study provided the information which is helpful to develop the official monograph of Canarium strictum Roxb.

ACKNOWLEDGEMENT

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collect plant parts and the resin from his herbal garden. We are grateful to Dr. Sivakumar General Manager, SKM Ayurveda, Siddha Pharmaceuticals, Erode for providing ancient palm script literature and encouragement all the way through the work.

**ABBREVIATIONS**

IUCN- International Union for Conservation of Nature, WHO- World Health Organization, QCMPMP- Quality control methods for medicinal plant materials, PARC- Plant anatomy research centre, Zn- Zinc, Mn- Magnesium, Cu- Copper, Cr- Chromium, Pd- Lead, UV- Ultraviolet, NBR- Nilgiri Biosphere Reserve, GCMS- Gas chromatography mass spectra, RLS- Radial longitudinal section, TLS- Transverse longitudinal section, FAA- Formalin Acetic acid Alcohol, TBA tertiary-Butyl alcohol, Cph- Collapsed phloem, NCPh- Non collapsed phloem, Pe- Periderm, SC- Secretary cavity, Sel- Sclereids, PhR- Phloem ray, ST- Sieve tube, Pa- Parenchyma, PCr- Prismatic crystal, Upright cells, Cr- Crystal, Fi- Fiber, PCr- Prismatic crystal, Sel- Sclereids, g- gram, μg- Microgram.

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