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Pharmacognostic investigations on *Bulbophyllum albidum* (Wight) Hook. f.

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Abstract: *Bulbophyllum albidum* (Wight) Hook. f. known to the *Kanikkars* as '*Kalmalpuloravi'* is an important medicinal plant. The *Kanikkar* tribe, inhabitants of Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu, India. use leaf and bulb of this plant to strengthening of a weak uterus for conception. The present investigation deals with the pharmacognostic studies of the leaf, stem, petiole and pseudobulb of the said plant. Pharmacognostic studies include microscopic, physicochemical constant (ash & extractive values), Fluorescence analysis and preliminary phytochemical evaluations.

Keywords: *Bulbophyllum albidum* (Wight) Hook. f., *Kanikkars*, Pharmacognosy, Fluorescence analysis.

INTRODUCTION

The practices of traditional medicine are based on hundreds of years of belief and observation, which predate the development of the modern medicine. In some countries traditional medicine remains as an integral part of the formal health system and exist on an equal footing with the current therapy. The method of practices of traditional medicine may appear to be numerous and dissimilar, but all represent variations of three basic activities, viz. faith healing, hygienic measures and drug therapy. Traditional medicine plays an important role in the health care of India. The recipes used in the traditional medicine of India have been handed down from the forefathers by oral tradition; however, these are disappearing from our modern society[1]. There is a need for documentation of research work carried out on traditional medicine. With this backdrop, it becomes extremely important to make an effort towards standardization of the plant material to be used as medicine. The process of standardization can be achieved by step wise pharmacognostic studies[2]. These studies help in identification and authentication of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproductive quality of herbal medicine which will contribute to its safety and efficacy. Simple Pharmacognostic techniques used in standardization of plant material include its morphological, anatomical and biochemical characteristics [3].

The *Bulbophyllum albidum* (Wight) Hook.f. belongs to the family Orchidaceae. It is commonly known as Kalmalpuloravi in *Kanikkar* tribals of Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu. *Kanikkar* tribe, use this plant for strengthening of a weak uterus for conception. However, perusal of literature reveals that Pharmacognostic information as *B. albidum* is totally lacking, hence in the present investigation was undertaken. The object of the present study is to evaluate various Pharmacognostic standards like microscopy of leaf ,stem, petiole and pseudobulp, ash values, extractive values, fluorescence analysis and preliminary phytochemical analysis of whole plant of *Bulbophyllum albidum*.

MATERIALS AND METHODS

Fresh plant materials were collected from the well grown plants found in the natural forest of Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu. Identification and confirmation were done by Botanical Survey of India, Southern circle, Coimbatore, Tamil Nadu, India. Where voucher specimens were deposited in the Ethnopharmacology unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin .

For anatomical investigations standard microtomy techniques[4] were followed. T.S. of 10 to 12 µm thickness were prepared. These microtome sections were stained with 0.25% aqueous Toluidine blue (Meta chromatic stain) adjusted to pH 4.7[5]. Photomicrographs were taken with Nikon trinocular photomicrographic unit. The most accepted descriptive terms were being used to describe the leaf and stem anatomy [6,7].

PHYSICOCHEMICAL AND FLUORESCENCE ANALYSIS

These analyses were carried out as per the standard procedures[8]. In the present study, the powdered leaf was treated with various chemical reagents like aqueous 1N Sodium hydroxide, alcoholic 1N sodium hydroxide, 1N hydrochloric acid, 50% sulphuric acid and concentrated nitric acid, picric acid, acetic acid, ferric chloride, conc.HNO_3 + NH_3 and their extracts were subjected to fluorescence analysis in day light and UV light (254 nm and 366 nm). Various ash types and extractive values were determined by following the standard methods[9].

PRELIMINARY PHYTOCHEMICAL ANALYSIS

Shaded dried and powdered leaf samples were successively extracted with Petroleum ether, benzene, chloroform and ethanol. The extracts were filtered and concentrated using vacuum distillation. The different extracts were subjected to qualitative tests for the identification of various phytochemical constituents as per the standard procedure [8,10].

RESULTS

ANATOMICAL STUDIES

LEAF

The leaf has smooth and even surfaces. It is 700 µm thick. The abaxial and adaxial epidermal layers are thin; the cells are narrowly oblong and have heavy cuticle. The epidermal cells are 30 µm thick. The stomata are located on the surface of the epidermis; however, the thick cuticle forms prominent beak-like stomatal ledge (Plate I).

The differention of the mesophyll tissue is less distinct. two or three layers of cells in the adaxial zone are vertically aligned simulating the palisade cells. Remaining portion of the mesophyll has about 10 layers of horizontally elongated less compact spongy parenchyma cells.

Table. 1. Ash and extractive values of the powdered whole plant of *Bulbophyllum albidum*

Ash Values

S.No	Type of Ash	% of Ash
1	Total ash value of powder	5.31 ± 0.03
2	Water soluble ash	0.90 ± 0.01
3	Acid insoluble ash	1.85 ± 0.03
4	Sulphated ash	6.62 ± 0.12

Extractive Values

S.No	Nature of extract	Extractive value (%)
1	Petroleum ether	5.40 ± 0.02
2	Benzene	3.25 ± 0.01
3	Chloroform	4.03 ± 0.02
4	Acetone	7.29 ± 0.11
5	Methanol	8.10 ± 0.03
6	Ethanol	6.78 ± 0.04
7	Water	12.26 ± 0.02

The vascular bundles of the viens occur in horizontal row along the median part of the lamina. No distinct midrib can be recognized. There is a large median vascular bundles and those bundles on either sides of the lamina are smaller. Both smaller and larger bundles are collateral with adaxial xylem and abaxial phloem. The xylem elements are small cluster and are angular and thin walled. The phloem strand is wide and massive with small darkly stained cells. The vascular strands are surrounded by one or two layers of parenchymatous cells which are smaller than the surrounding mesophyll cells.

Raphides: Calcium oxalate needles aggregated into prominent bundles are often seen in the mesophyll tissue. They occur in unmodified parenchyma cells. The raphide bundle is 70 µm long and 30 µm thick .

Mucilage is diffusely distributed in the leaf cells. It is filled within the epidermal cells and also occur in the mesophyll.

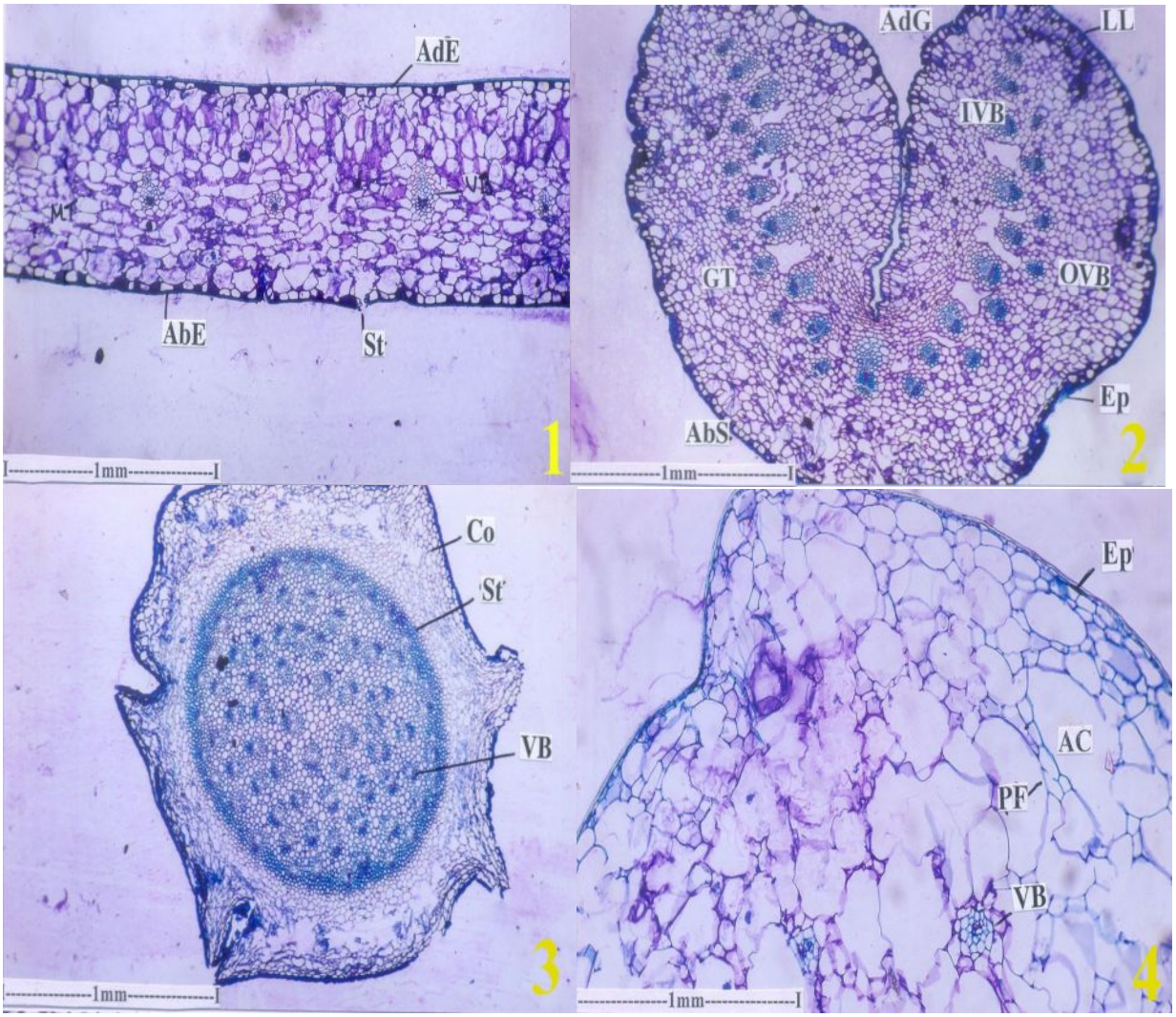
Table. 2. Fluorescence analysis of the powdered whole plant of *Bulbophyllum albidum*

Experiments	Visible / Day light	UV Light	
		254nm	365nm
Drug powder as such	Brown	Brown	Brown
Powder + 1N NaOH (aqueous)	Yellow	Greenish yellow	Green
Powder + 1N NaOH (alcohol)	Yellow	Greenish yellow	Green
Powder + 1N HCL	Yellow	Pale yellow	Pale green
Powder + 50% H_2So_4	Yellow	Pale yellow	Pale green
Drug powder + Nitric acid	Brown	Fluorescent green	Brown
Drug Powder + Picric acid	Yellow	Fluorescent green	Brown
Drug Powder + Acetic acid	Brownish yellow	Greenish yellow	Reddish brown
Drug Powder + Ferric chloride	Yellow	Fluorescent green	Brown
Drug Powder + HNO_3 + NH_3	Brown	Yellow	Green

Table. 3. Preliminary phytochemical screening of whole plant extracts of *Bulbophyllum albidum*

Test	Petroleum ether	Benzene	Chloroform	Ethanol
Alkaloid	-	+	+	+
Anthraquinone	-	-	-	-
Catachin	+	-	-	+
Coumarin	-	+	+	-
Flavonoid	-	-	-	+
Phenol	+	-	-	+
Quinone	-	-	-	-
Saponin	-	-	+	+
Steroid	+	-	-	-
Tannin	+	+	-	+
Terpenoid	-	-	+	+
Sugar	-	+	+	+
Glycoside	+	-	-	+
Aminoacid	-	-	+	+
Xanthoprotein	-	-	+	+
Fixed oil	+	-	+	-

PLATE I



1. T.S. of lamina through vascular trace
2. T.S. of petiole – entire view
3. T.S. of stem – entire view
4. T.S. of the bulb - a sector enlarged

AbE – Abaxial epidermis; AdE – Adaxial epidermis; MT – Mesophyll tissue; VB – Vascular bundle; AbS - Abaxial side; AdG – Adaxial groove; AC – Air chamber; Co – Cortex; Ep – Epidermis; GT – Ground tissue; IVB – Inner vascular bundle; LL – Lateral lobe; OVB – Outer vascular bundle; St - Stele; PF – Partition filament

PETIOLE

The petiole is semicircular in sectional view; with deep, narrow canal-like adaxial groove (Plate I, 2). It is 2 mm thick vertically and 2.3 mm wide horizontally. The epidermal layer is prominent comprising of radially oblong cells with thick cuticle, The ground tissue consists of angular, compact parenchyma cells. Fairly wide air-chambers of lysigenous origin, are seen along the border of the adaxial canal and inbetween the vascular bundles. Mucilage is seen in amorphous masses in the epidermis and ground tissue.

The vascular system consists of two bowl- shaped lines, one towards the inner part and the other on the outer part. The inner vascular bundles are larger than the outer bundles. The numbers of bundles in both lines are numerous and they are collateral. The vascular bundles have inner, small cluster of xylem elements and outer wider mass of phloem elements. A thick layer of bundle sheath fibres of thick lignified walls surrounds the vascular bundles. The fibre-sheath is wider in the phloem mass than in the xylem part.

STEM

The stem has uneven outline due to the persistent leaf-scars (Plate I, 3). It is 1–7 mm thick. The epidermis is thin with elliptical cells which have heavy cuticular layer. The cortex is narrow, homogenous and parenchymatous. The cells are thin walled and compressed. Major portion of the stem is occupied by a wide circular atactostele. The endodermis is not well defined. There is a thick cylinder of sclerenchyma cells, which can not be easily differentiated from endodermal layer. The vascular bundles are many and diffusely distributed in the pith. A ring of vascular bundles are immersed in the inner boundary of the sclerenchyma cylinder; others are in the central part. All the bundles are collateral; the phloem units of all the bundles are directed towards the periphery. The stellar cylinder is 1.2 mm in diameter.

The vascular bundles that are associated with the sclerechyma cylinder have small nests of phloem and a short radial row of wide angular thin walled xylem elements. The central bundles are larger and circular. They have larger mass of phloem and circular cluster of thick walled angular xylem elements. The vascular bundles are surrounded by a thin layer of fibres; the fibre – sheath is wider in the region of phloem than other regions. The ground tissue is parenchymatous; the cells are circular, thick walled and are loosely arranged.

PSEUDOBULB

The Pseudobulb has a thin epidermal layer and the epidermal cells are very narrow and cylindrical. The ground tissue is aerenchymatous. There are wide air-chambers of irregular shape and size separated by thin uniseriate shape and partitions (Plate I, 4). The cells have large masses of mucilage, scattered in the aerenchymatous ground tissue are seen several discrete vascular bundles. The vascular bundles are of two types: some are smaller and circular having a few xylem elements and prominent mass of phloem elements. The larger bundles have a flat band of wide, thick-walled angular xylem elements and two wide masses of phloem separated by a parenchymatous gap. There is no sclerenchymatous bundle-sheath.

Raphide bundles are more abundant in the pseudobulb. The bundles are short and thick. They are located within ordinary parenchymatous ground tissue. The raphides are 50 μm long and 25 μm thick.

POWDER ANALYSIS OF THE DRUG

The results of the ash and extractive values of whole plant of *Bulbophyllum albidum* drug powder are depicted in Table - 1. The total ash content of the powdered whole plant is 5.31% and extractive value in water is more than in other solvents. The results of fluorescent analysis of whole plant powder of *Bulbophyllum albidum* are shown in Table - 2. The leaf powder shows the characteristic flurosecent green colour treated with nitric acid, picric acid and ferric chloride. The result of preliminary phytochemical screening of whole plant extracts of *Bulbophyllum albidum* are presented in Table - 3. The ethanol extracts of the whole plant shows the presence of alkaloid, catachin, tannin, saponin, flavonoid, phenol, terpenoid, sugar, glycoside and xanthorpotein.

DISCUSSION

The present study attempts a modest comprehensive investigation of the whole plant of *Bulbophyllum albidum*. Since the whole plant of *Bulbophyllum albidum* as the folklore claims has therapeutic qualities the present investigation has laid down a set of anatomical features of the leaf, petiole, stem and pseudobulb which can be employed for its botanical diagnosis. The salient features of identification of the fragmentary sample are;

- Leaf is smooth and even on both sides; lamina isolateral without distinction between adaxial and abaxial sides.
- Epidermal layers with rectangular cells and thick cuticle.
- Mesophyll tissue undifferentiated into palisade and spongy parenchyma cells; 10 – 12 layers of polygonal, less compact thin walled parenchyma cells.
- Stomata with prominent, beck-like cuticular ledges.
- Vascular strands occur in a horizontal single row of smaller and larger bundles.
- Foliar bundles are collateral with adaxial xylem and abaxial phloem, bundles surrounded by thick parenchymatous bundle sheath.
- Raphide bundles of calcium oxalate frequently in the mesophyll.
- Stem is circular with shallow wide furrows. Cortex wide and parenchymatous; cortical bundles absent. Stele with sclerotic endodermoid layer and thick band of sclerotic cylinder.
- Steler bundles form atactostele with collateral, angular wide metaxylem elements and small cluster of phloem.
- Petiole is semicircular in cross section outline with deep, narrow adaxial groove`.
- It has prominent epidermis with cuticle, parenchymatous ground tissue and discrete vascular bundles arranged in two, separate U shaped outline.
- The bundles in the outer line are slightly larger than those in the inner line.

- The vascular bundles are collateral with sclerenchyma bundles sheath, cells around the phloem are more thick walled than other side.
- Pseudobulb has thin less prominent epidermis with thick cuticle.
- The ground tissue is parenchymatous and thin walled with wide air-chambers scattered throughout.
- Mucilage is abundant in the ground tissue.
- The vascular system consists of several discrete scattered vascular strands.
- Some of the vascular strands are smaller with a few wide angular xylem and a thick band of phloem. Others are larger with wide arc of xylem and thick mass of phloem.
- The vascular strands have no well defined bundle sheath.
- Raphides are frequently seen in the ground tissue.

The physical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs[11]. Equally important in the evaluation of crude drugs, is the ash value and acid insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e., the presence or absence of foreign organic matter such as metallic salts and/or silica[12]. The ash value of whole plant of *Bulbophyllum albidum* is 5.31%. This ash value is indicative of the impurities present in the drug. Since the ash value is constant for a given drug, this value is also one of the diagnostic parameters of the drug. In the present study, whole plant of *Bulbophyllum albidum* has more water soluble ash than acid insoluble ash. The ash value is generally the index of the purity as well as identity of the drug.

Many phytocompounds fluoresce when suitably illuminated. The fluorescence colour is specific for each compound. A non fluorescent compound may fluoresce if mixed with impurities that are fluorescent. The fluorescent method is adequately sensitive and enables the precise and accurate determination of the analyze over a satisfactory concentration range without several time consuming dilution steps prior to analysis of pharmaceutical samples [13].

In the present study, the powdered whole plant of *Bulbophyllum albidum* emitted brown under day light, short UV and long UV light respectively. Presence or absence of certain important compounds in an extract is determined by colour reactions of the compounds with specific chemicals which act as dyes. This procedure is a simple preliminary pre-requisite before going for detailed photochemical investigation. Various tests have been conducted qualitatively to find out the presence or absence of bioactive compounds. Different chemical compounds such as alkaloid, catachin, tannin, saponin, flavonoid, phenol, sugar, glycoside and xanthoprotein are detected in whole plant of *Bulbophyllum albidum* extracts, which could made the plant useful for treating different ailments as having a potential of providing useful drugs of human use.

Saponins, a group of natural products occur in the whole plant of *Bulbophyllum albidum* extracts. In plants, the presence of steroidal saponins like, cardiac glycosides appear to be confined to many families and these saponins have great pharmaceutical importance because of their relationship to compounds such as the sex hormones, cortisones, diuretic steroids, vitamin D etc.,[14]. Saponin reduce the uptake certain nutrients including glucose and cholesterol at the got through intra-luminal physicochemical interactions. Hence, it has been reported to have hypocholesterolemic effect and thus may aid lessening metabolic burden that would have been placed in the liver [15].

Several authors reported that flavonoids, sterols/terpenoids, phenolic acids are known to be bioactive antidiabetic principles [16,17]. Flavonoids are known to regenerate the damaged beta cells in the alloxan induced diabetic rats [18]. Flavonoids act as insulin secretagogus [19]. Most of the plants have been found to contain substances like glycosides, alkaloids, terpenoids, flavonoids etc, which are frequently implicated as having antidiabetic effects [20].

Since the plant, *Bulbophyllum albidum* is useful in traditional medicine for the treatment of various ailments, it is important to standardize it use for as a drug. The Pharmacognostic study of the *Bulbophyllum albidum* has been carried out for the first time. The pharmacognostic constant for the various parts of above said plant, the diagnostic microscopic features and the numerical standards reported in this work could be useful for the compilation of a suitable monograph for it proper identification.

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