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Full Length Research Paper

Pharmacognostical standardization of *Commiphora berryi* (Arn) Engl and phytochemical studies on its crude extracts

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Commiphora genus of Burseraceae family comprises of more than 175 species. Among them many species have been reported with diverse medicinal potential. *Commiphora berryi* (Arn) Engl is a member of this genus and has been reported to have potential use in folklore medicine to treat various ailments such as ulcer, infection, loss of appetite etc. To supplement the necessary information for the systematic identification and authentication of this particular species, pharmacognostic standardization of various parts of this plant as per WHO guidelines and phytochemical studies on various crude extracts obtained from the stem bark of this plant were carried out and reported.

Key words: *Commiphora berryi*, burseraceae, pharmacognostical standardization, phytochemical studies, TLS, LS, RLS.

INTRODUCTION

For centuries, plants and plant products have been used for treating various illnesses. Several medicinal plants and their products are still in use either as home remedies or as over-the-counter drugs and they represent a substantial proportion of the global drug market. However, standardization of natural products is a complex task due to their heterogeneous composition, which is true for, either the whole plant, or any plant part or extracts obtained thereof. To ensure reproducible quality of herbal products, authentication of the starting material is essential. According to WHO (1988), the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken.

Balsomodendron berryi (Arn) Bedd, currently known as *Commiphora berryi* (Arn) Engl, (Tamil name: Mudgiluvai, Family: Burseraceae) is a small fragrant, thorny tree occurring in the dry forests of North Coimbatore hills and is also commonly grown as hedge plant throughout South India. It yields a fragrant gum resin obtained by incision of the bark. The resin is used in folklore medicine as an ingredient in multi-component indigenous formulations used as astringent, antiseptic, carminative, diuretic, appe-tite stimulator, uterine stimulant and emmenagogue.

The resin has also been used as a fixative in perfumery

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Abbreviations: **Abe** , Abaxial epidermis; **Abs** , abaxial side; **Ade**, adaxial epidermis; **Ads**, adaxial side; **Ap**, axial parenchyma; **Co**, cortex; **CPh**, collapsed phloem; **Cr**, crystals; **Cz**, cambial zone; **EC**, epithelial cell; **En**, endocarp; **Ep,** epidermis; **Ec**, epicarp; **GC**, granular contents; **GT**, ground tissue; **Hsc**, horizontal secretory canal; **IZ**, inner zone; **La**, lamina; **LV**, lateral vein; **MC**, Mesocarp; **MR**, midrib; **NCPh**, non-collapsed phloem; **OZ**, outer zone; **Pc**, procumbent cell; **Php**, phloem parenchyma; **Ph**, phloem; **PhR**, phloem ray; **PhR**, phloem ray; **PL**, placenta; **Pm**, pallisade mesophyll; **Sc** , subsidary cells; **Sc**, sclereid; **Sc**, sclerenchyma; **Sc,** secretory canal; **SC,** secretory cavity; **SM**, spongy mesophyll; **Sp**, sieve plate; **STM**, sieve tube member; **ST**, seive tube; **St**, stomata; **SX**, secretory xylem; **TT,** terminal tracheid; **UC**, upright cells; **VB**, vascular bundle; **Vi**, vein islets; **VS**, vascular strand; **VT**, vein termination; **X**, xylem.

Figure 1. Photograph of *Commiphora berryi* (Arn) Engl in its natural habitat.

Figure 2. Two branches showing com-pound leaves and spines.

and as an adulterant for other oleoresins (Anonymous, 1950; Brandis, 1970). Diuretic activity of petroleum ether extract of *C. berryi* bark and some of its isolated fractions (Selvamani et al., 2004a, b) and antibacterial activity of *C. berryi* leaves have been reported (Latha et al., 2005). Only one evidence of phytochemical study done so far on the bark of *C. berryi* is found (Gowrishankar et al., 2004). Thus, as there was ample scope to work on this plant for various pharmacognostic parameters, we, in this present study, have tried to provide comprehensive information on microscopic and morphological characters of the leaves, bark and fruits and other pharmacognostic parameters of *C. berryi*.

MATERIALS AND METHODS

Pharmacognostic studies

Description of *Commiphora berryi* (Arn) Engl: The plant is profusely branched deciduous tree growing up to 10 m in height (Figure 1). The leaves are 3 - 7 foliolate, alternate, leaflets opposite (Figure 2) with inflorescence axillary panicle. Flowers are tetramerous, sepals are four, gamosepalous, companulate, per-sistent and valvate while petals are four and disc- cupular. Stamens are 8 unequal and anthers oblong, ovary-2 celled with two ovules in each cell; style-short, stigma lobed; fruit-drupe and ovoid-subglobose.

Collection of specimens

The plant specimens for the proposed study were collected from Tirunelveli district of Tamilnadu. The specimens were identified and authenticated by Botanical Survey of India, Tamilnadu Agricultural University, Coimbatore, Tamil Nadu, India (Ref. BSI/SC/5/21/04-05/TECH-1752 Dt. 4th January, 2005) . Care was taken to select healthy plants and for normal organs. The leaf, petiole, bark and fruit were cut and removed from the plant and fixed in formalin acetic acid solution (formalin:acetic acid:70% ethyl alcohol in the ratio of 0.5:0.5:9). After 24 h of fixation, the specimens were dehydrated with graded series of tertiary-Butyl alcohol as per the schedule given by Saa, 1940. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58 - 60°C) until thiobarbituric acid solution attained super saturation. The specimens were then casted out into paraffin blocks.

Preparation of sections

The paraffin embedded specimens were sectioned with the help of a rotary microtome. 10 - 12 µm thickness of the sections was made. However, dewaxing of the sections was done using customary procedure (Johansen, 1940). The sections were later stained with toluidine blue as per the method published by O'Brien et al. (1964). Since, toluidine blue is a polychromatic stain, the staining was remarkably good and yielded varied cytochemical reactions. The dye rendered pink color to the cellulose walls, blue to lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. However, where necessary, sections were also stained with safranin, fast-green and iodine-potassium iodide for starch.

In studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections taken parallel to the surface of leaf were cleared with 5% sodium hydroxide and epidermal peeling was prepared through partial maceration by employing Jeffrey's maceration fluid (Sass, 1940). Cleared sections were then mounted with glycerine for microscopical observation. Moreover, powdered materials of different parts of the plant were also cleared with NaOH and mounted in glycerin medium after staining. In addition, the different cell components were later studied and reported.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photomicrographs of different magnifycations were taken with Nikon Labphot 2 microscopic unit. For normal observation, a bright field microscopy was used and for the study of crystals, starch grains and lignified cells, a polarized light was employed. However, since these structures have birefringent property, under polarized light they tend to appear bright against the dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as per the standard anatomy books (Esau, 1964).

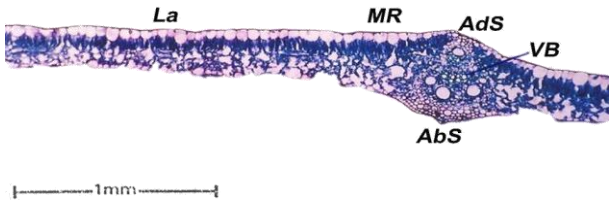

Figure 3. Anatomy of the leaf –TS of midrib with lamina.

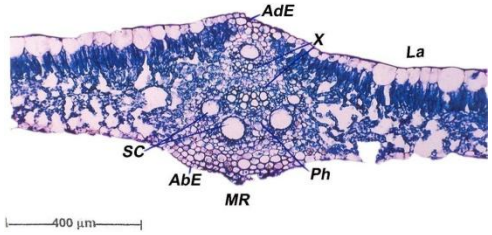

Figure 4. Anatomy of the leaf –TS of midrib (enlarged).

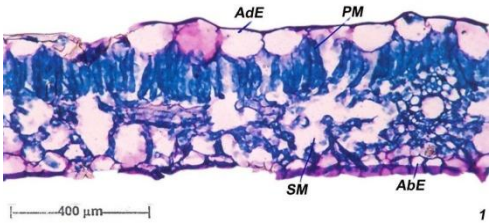

Figure 5. Structure of lamina – TS of Lamina through lateral vein.

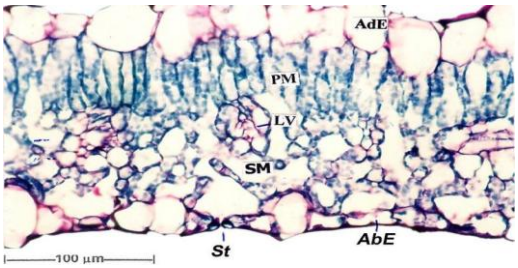

Figure 6. Structure of lamina – TS of lamina through lateral veins (enlarged).

RESULTS AND DISCUSSION

Anatomy of leaf

The leaf is dorsiventral, hypostomatic, mesomorphic and glabrous on both sides; its surface is even and smooth (Figure 3). The midrib is slightly raised on both adaxial and abaxial sides and spindle shaped in sectional view. It is 420 µm in vertical plane. The epidermal layer becomes thinner in the midrib region; the cells are small and squarish as shown in Figure 4. There is a parenchymatous ground tissue which extends from the abaxial part to the adaxial part. The vascular system consists of a median short arc of xylem and narrow zone of phloem. There is also an adaxial accessory, small vascular bun- dle. Adjacent to the xylem, there are three wide circular secretory canals placed in a row; the adaxial bundle also has a secretory canal just above the xylem strands (Figure 4).

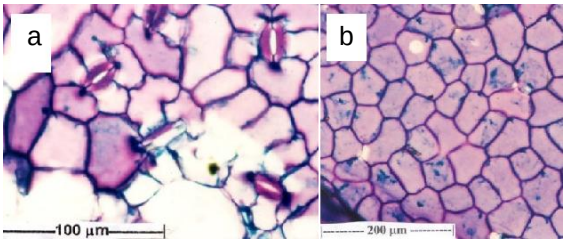

Figure 7a and b. Surface features of adaxial epidermis.

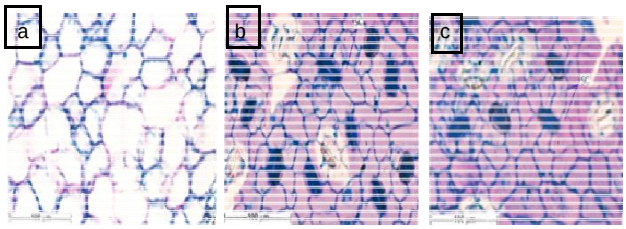

Figure 8a, b and c. Surface features of adaxial epidermis with stomata.

Lamina

The lamina is about 320 µm thick. It has wide adaxial epidermal layer made up of dilated squarish thin walled cells as shown in Figure 5 and a thin wall abaxial epidermal layer of narrow tubular cells. However, the adaxial epidermis is stomatiferous (Figure 6). The mesophyll is divided into adaxial with a single row of palisade cells and abaxial spongy parenchyma zone. The palisade zone has short cylindrical less compact cells measuring about 110 µm in height. The spongy mesophyll has about 8 layers of lobed network of spongy mesophyll tissue.

Surface features of the epidermis tissue

The adaxial epidermis has polyhedral thick walled cells, the auticlinal walls are straight, cuticular striations are not evident (Figures 7a and b). The abaxial epidermis is stomatiferous. The stomata are actinocytic, which is, surrounded by six to seven radically oblong cells. The epidermal

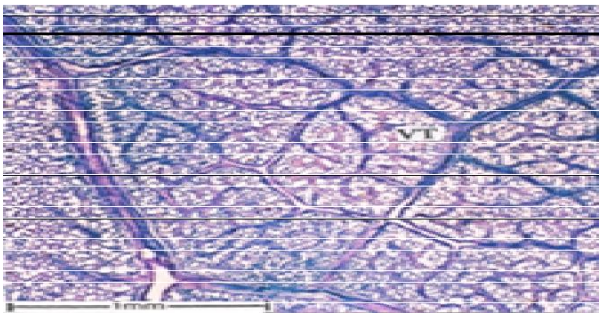

Figure 9. Venation pattern of *Commiphora berryi* (Arn) Engl.

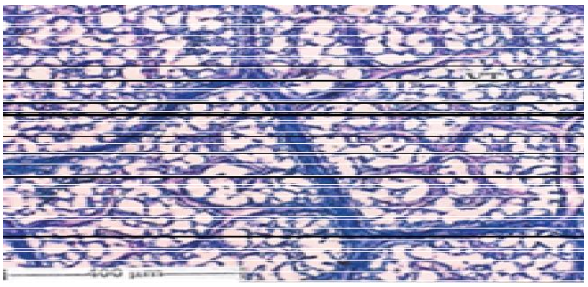

Figure 10. Venation pattern – Vein islets and vein termination (enlarged).

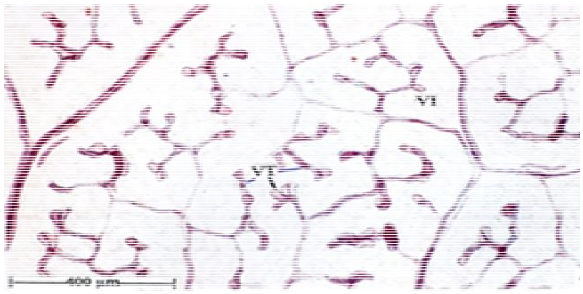

Figure 11. Cleared leaf showing vein islets and vein termination.

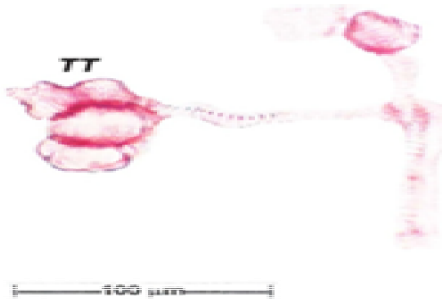

Figure 12. Cleared leaf showing vein islets and vein termination (enlarged).

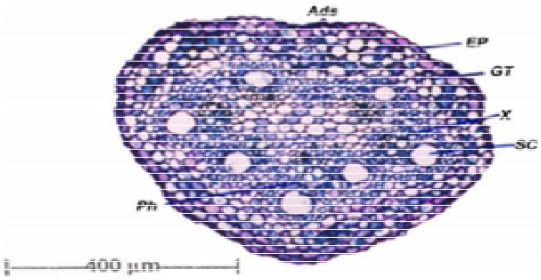

Figure 13. Anatomy of the petiole – TS of petiole (distal region) entire row.

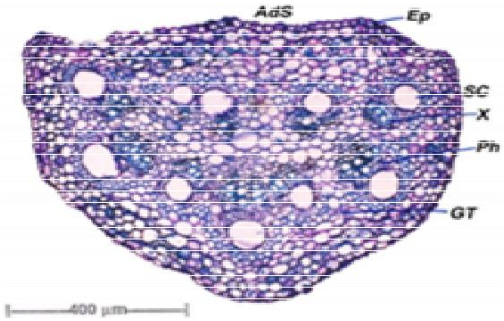

Figure 14. Anatomy of the petiole – TS of petiole (proximal region) ground plan.

usually has more than one vein termination; the termanatious branch repeatedly forming deudroid outline (Figures cells are polygonal; their auticlinal walls are thick and straight (Figures 8a, b and c). The guard cells are elliptical and measure 30 - 35 x 20 - 25 µm.

Venation pattern

The vein lets are fairly thick. They form distinct vein lets, which are wide and polygonal in outline. Each vein islet 9 and 10). The ultimate endings of the vein terminations are characterized by the presence of a cluster of terminal tracheids (tracheoids); the tracheids are spherical or irregularly lobed (Figures 11 and 12).

Petiole (Rachis)

The rachis of the compound leaf is more or less circular (Figure 13) or dorsiventral (Figure 14). The distal end of the petiole, which is circular and slightly wider in tangential plane measuring 500 µm; along the vertical plane, it is 450 µm. The proximal part of the petiole is adaxially flat and abaxially semicircular (Figure 14). It is 600 µm in

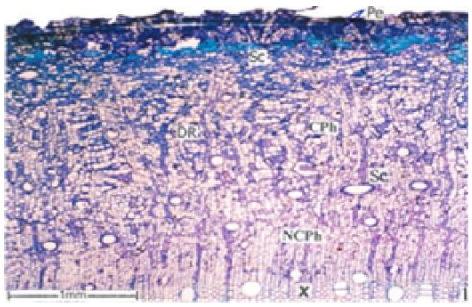

Figure 15. Gross microscopic features of the bark and crystal distribution – TS of bark entire view.

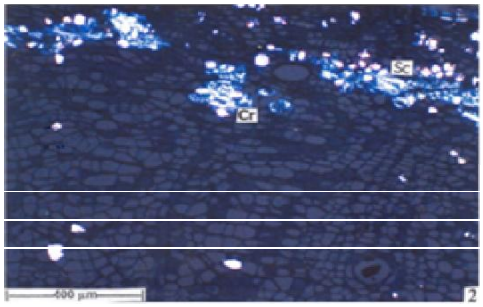

Figure 16. Periderm and secondary phloem under high magnification.

sclernchymatous cells (Figures 13 and 14). The bundles are separated from each other by narrow parenhymatous rays.

Anatomy of bark

The bark is 1.8 mm thick. It has smooth surface with membraneous exfoliating flakes. The bark shows the following tissue zones:

i.) Periderm: it is superficial and less prominent (Figure 15 and 16). It measures 50 µm thick at certain regions and 150 µm in outer regions. The cells are narrowly oblong vertical plane and 800 µm in horizontal plane. The epidermal layer of the petiole is thin and the epidermal cells have papillate outer tangential walls. The ground tissue consists of small polygonal or circular compact parenchyma cells. The cells along the periphery are smaller and those in the cube are larger. The vascular system consists of 9 to 10 discrete vascular bundles which are arranged in a ring. Each bundle is collateral with inner xylem, outer phloem and with a wide circular secretory canal which is covered by an arc of oblong and thick walled. The superficial cells break away as thin membrane.

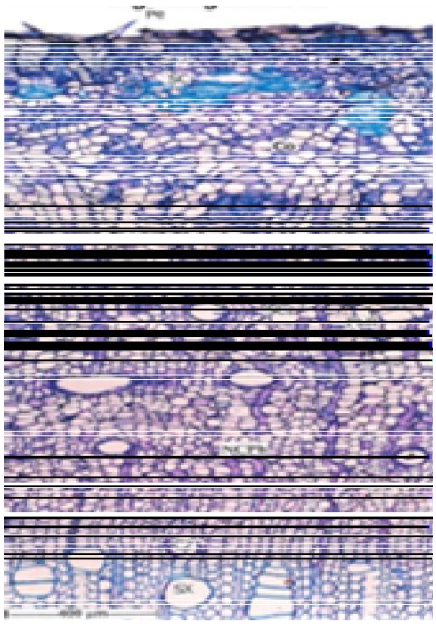

Figure 17. Secondary phloem tissues and secondary canal – Collapsed and non-collapsed phloem and tangential band of secretory canals.

ii.) **Screnchyma cylinder:** Inner to the periderm, there is discontinuous cylinder of thick walled sclereids with wide lumen.
iii.) **Cortex:** It is formed partly by the original remnant of the cortical parenchyma and partly by dilated phloem ray parenchyma cells (Figure 16). The cortical cells are tangentially elongated and have undergone frequent radial divisions. Thus they are in tangential clusters of compactly arranged wide zone.
iv.) **Secondary phloem:** it is the major portion of the bark. It consists of two zones, namely outer collapsed phloem and inner non collapsed phloem.

Collapsed phloem

It consists of wide undulated phloem rays, dilated compact parenchyma cells, wide circular secretory canals and crushed sieve elements which are visible in dark thin streaks. The cells are not in radial alignment. The collapsed phloem is nearly 1.4 mm wide.

Non collapsed phloem

It is located outer to the cambial zone (Figure 16). It has thin straight phloem rays, intact sieve elements and small parenchyma cells. All these cells are in regular parallel radical rows. The sieve elements are horizontally rectangular and the parenchyma cells are polygonal (Figure 17).

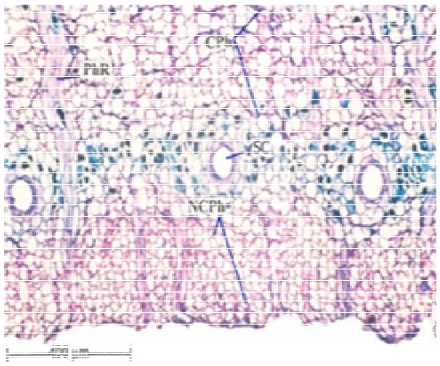

Figure 18. Secondary phloem tissues and secondary canal (Enlarged).

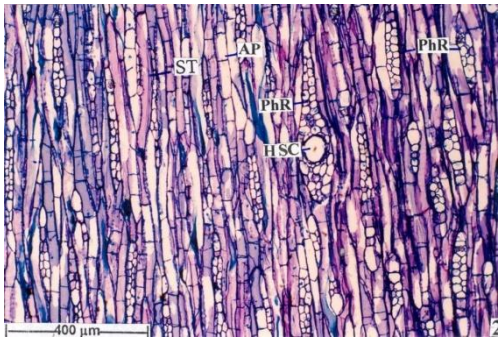

Figure 19. TLS view of bark showing anastomosting secretory canal as seen in the secondary phloem of TLS view.

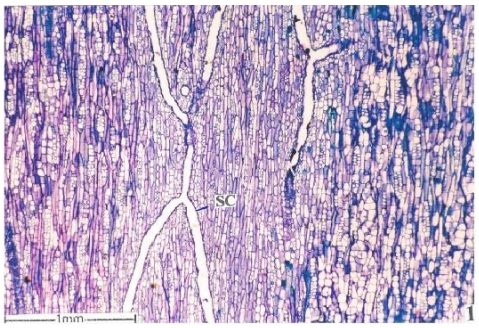

Figure 20. TLS of phloem showing non storied phloem ray and spindle shaped ray and secretory canal.

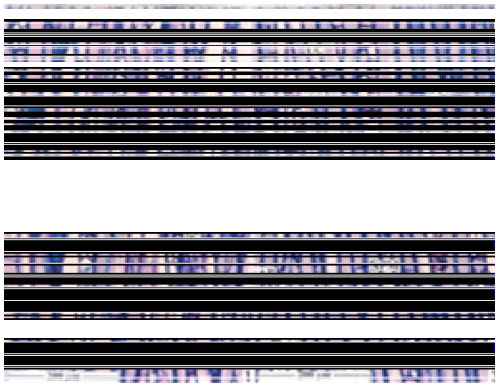

Figure 21. TLS of phloem showing ray seriation and sieve tube membrane.

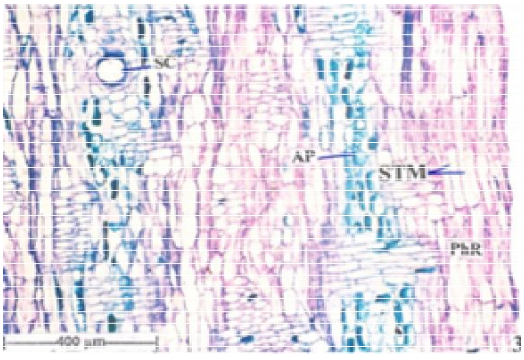

Figure 22. RLS view of phloem.

Secretory canal

These are wide, circular vertically running canals, found in the collapsed phloem. The canal is enclosed by a layer of densely staining epithelial cells (Figure 18). It is 70 µm in diameter.

Phloem in TLS and RLS sectional view

The structure and organization of the phloem rays, sieve elements and axial parenchyma cells were studied in LS view. The phloem rays are nonstoried but long and narrow. The vertical secretory canals are non articulated and anatomosing type (Figure 19) and are vertical and oblique. The phloem rays are predominantly biseriate and heterocellular. They consist of central biseriate portion and marginal portion with one or two, wide upright cells. The central procumbent cells are polygonal in TLS view (Figure 21) or horizontally elongated rectangular cells in RLS view (Figure 22). Some of the phloem rays have horizontal secretory canal, placed in the midportion; such rays become multiseriate and are celled fusiform rays (Figure 20). The rays are 200 - 300 µm in height and 30 - 40 µm wide. Ray frequency is 7 mm. Seive tube members are narrow and long. The sieve plate is simple and oblique. They are 370 - 480 µm in height and 20 - 30 µm wide. Their walls are thick (Figure 21) . Axial parenchyma cells are vertically running cells. They occur in vertical strands. They are narrow and rectangular in shape (Figure 21).

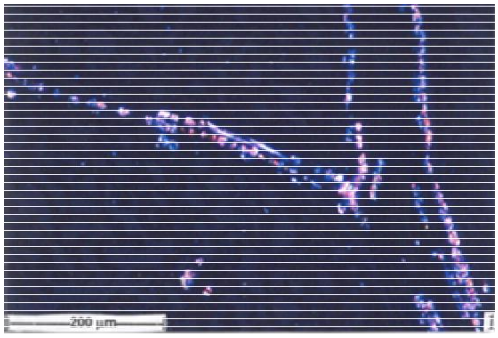

Figure 23. Crystal distribution showing along the vein cells in powder (under polarized light microscope).

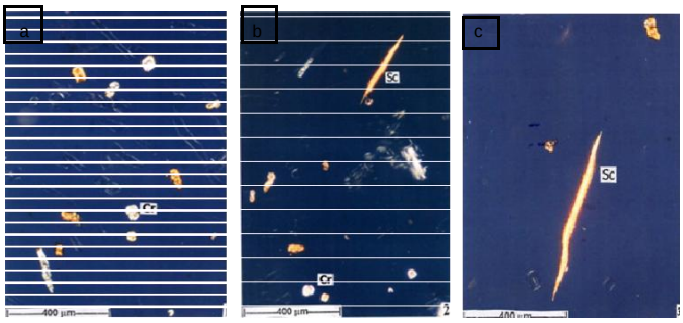

Figure 25a, b and c. Powder microscopy of the bark – Crystals and schlereids (Under polarized light microscope).

Powder microscopy

In the powder, leaf fragments and bark elements were observed. Small fragments of the lamina with veins, when viewed under the polarized light microscope, exhibited dense accumulation of calcium oxalate crystals along veins (Figure 23 and 24). Prismatic crystals were also seen mixed with the druses. The druses are 10 - 12 µm wide. Crystals were also seen in the bark; they were prismatic and located along the sclerenchyma bands. Bark powder shows scattered calcium oxalate druses and sclerids (Figure 25a, b, c and 26) . Prismatic crystals were also observed. The sclerids are of two types. Some of these are elongated with tapering ends (Figure 25c). These are called fiber sclereids. They are up to 470 µm long. The branchy sclereids are elongated, rectangular cells (Figure 26). They have wide lumen and thick walls with narrow pits.

Anatomy of fruits

The fruit of *C. berryi* is ovoid-elliptical with a pointed beak at the apex. It is reddish brown with smooth surface. It is 1.2 cm long and 6 mm broad (Figure 27). In cut-view, the outer part is brown and the inner part is white with lateral

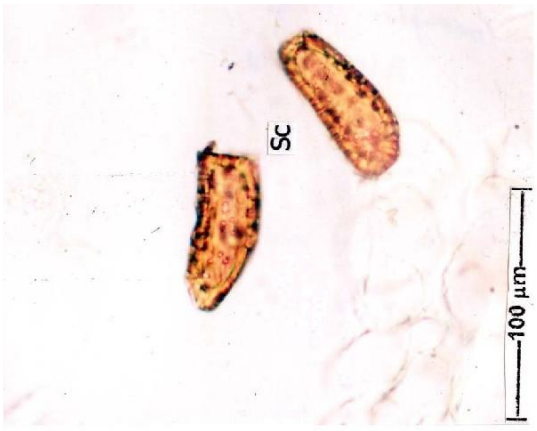

Figure 26. Powder microscopy of the bark – branchy sclereid in the bark powder under bright field microscope.

Figure 27. Branch of *Commiphora berryi* (Arn) Engl bearing fruits.

protruding placental funicular tissue. The fruit has central hollow wide cavity. In trans-sectional view, the fruit is roughly circular in outline with shallow furrows at four places (Figure 28). The fruit is a soft drupe differentiated into epicarp, mesocarp and fleshy endocarp. Total thickness of the pericarp is 1.3 mm. On drying the endocarp gets separated from the epicarp–mesocarp zones. These three, fairly wide tangentially stretched canals, one is the placental tissue, the other two on either side of the placentum. Epicarp is represented by thin, less conspicuous, small, thick walled epidermal cells with prominent cuticle (Figure 29). Inner to the epicarp is the wide mesocarp which is 850 µm wide. It is differentiated into outer narrow zone of 100 µm wide, thick walled darkly staining compact cells, the inner zone has wide, thin walled radially elongated parenchyma cell layers. Small vas-cular strands are seen along the inner boundary of the parenchyma cell zone. In the outer part of the paren-

Table 1. Analytical parameters for stem bark of *Commiphora berryi* (Arn) Engl.

S. No.	Parameters	Results
1.	Powder fineness /sieve size	Moderately coarse (710/250)
2.	Foreign matter	None
3.	Acid insoluble ash	1.43
4.	Total ash	8.96
5.	Water soluble ash	0.92
6.	Loss on drying	8.31
7.	Bitterness value	Mild
8.	Heavy metals	
	Arsenic:	0.27 ppm
	Cadmium:	0.04 ppm
	Lead:	Not detected
	Mercury:	0.05 ppm
9.	Foaming index	<100
10.	Swelling index	3.33

Table 2. Behavior of the *Commiphora berryi* (Arn) Engl bark powder.

S. No.	Procedure	Observation	
		Under normal light	Under UV light
1.	Powder as such	Reddish brown	Yellowish green
2.	Powder + Saturated solution of picric acid	Yellowish red	Reddish orange
3.	Powder + Nitric acid	Dark reddish orange	Dark green
4.	Powder + Hydrochloric acid	Brown	Black
5.	Powder + 80% Sulphuric acid	Dark brown	Light green
6.	Powder + Glacial acetic acid	Light brown	Bright green
7.	Powder + 5% Ferric chloride solution	Brown	Dark brown
8.	Powder + 5N Sodium hydroxide Solution	Dark brown	Yellowish green
9.	Powder + Aqueous Iodine solution	Reddish brown	Yellowish green

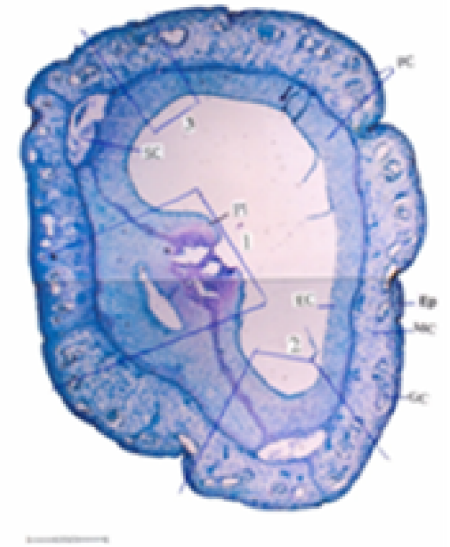

Figure 28. Median TS of fruit of showing epicarp, mesocarp and endocarp. Sector - 1. Placental tissue of the fruit; Sector - 2. Pericarp showing secretory cavities with granular contents and vascular strands.

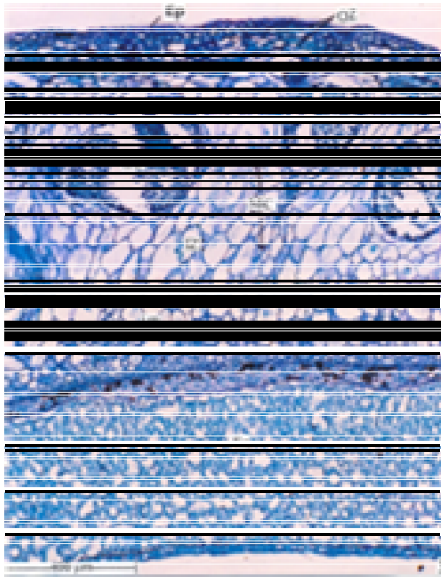

Figure 29. Sector - 3 of Figure 28- enlarged showing epicarp, mesocarp with granular cavities and vascular bundles, the endocarp has spongy lobed parenchyma cells.

Table 3. Extractive values for various solvents for the stem bark of *Commiphora berryi* (Arn) Engl.

S.No.	Solvent	Extractive value (% w/w)
1.	Water	12.76
2.	Acetone	2.15
3.	Methanol	11.82
4.	Ethanol	9.99
5.	Butanol	10.06
6.	Ethyl acetate	3.50
7.	Dichloromethane	1.85
8.	Chloroform	2.15
9.	Carbon tetrachloride	1.40
10.	Petroleum ether (40-60˚C)	2.32
11.	Benzene	1.66
12.	Hexane	2.53

Table 4. Phytochemical analysis of various extracts of *Commiphora berryi* (Arn) Engl.

Phytoconstituents	W	A	M	E	Bu	EA	DCM	C	CT	PE	Be	Hex
Alkaloids	-	-	-	-	-	-	-	-	-	-	-	-
Amino acids	-	-	-	-	-	-	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-	-	-	-	-	-	-
Flavonoids	-	-	+	+	+	-	-	-	-	-	-	-
Glycosides	+	+	+	+	+	-	-	-	-	-	-	-
Proteins	-	-	-	-	-	-	-	-	-	-	-	-
Reducing sugars	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	+	+	-	-	-	-	-	-	-	-	-	-
Starch	+	+	+	+	+	-	-	-	-	-	-	-
Steroids	-	-	+	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	-	-	-	-	-	-	-
Terpenoids	-	-	+	+	+	+	+	+	+	+	+	+

W-Water, A-Acetone, M-methanol, E-Ethanol, Bu-Butanol, EA-Ethyl acetate, DCM-Dichloromethane, C-Chloroform, CT-carbon-tetrachloride, PE-Petroleum ether, Be-Benzene, Hex-Hexane.

chyma zone occur wide cavities filled with small darkly staining granular contents. The inner boundary of the mesocarp consists of two to three layers of small narrowly rectangular cells forming dark line. The endocarp has an outer layer of rectangular, narrow rectangular cells and an inner boundary of similar cell layer. In between these layers is a wide zone of highly lobed cells which are connected with large air-spaces. The endocarp appears spongy in appearance.

Analytical parameters

As the authors are mostly involved in a thorough investigation on the bark component of the plant at present, experimental data provided are mainly bark related. However, various analytical parameters include powder fineness, sieve size, foreign matter, ash values, water content, swelling index, foaming index, heavy metals and radioactive contamination in the raw materials were determined by following the WHO guidelines (Anonymous, 1998) and reported in Table 1. Changes of bark powder behavior with different reagents under normal and UV lights was observed and reported in Table 2.

Extractive values

The collected bark was shade dried and powdered. 100 gm of the freshly powdered bark was extracted exhaustively with previously distilled solvents in a soxhlet apparatus for 36 h and the excess solvent was removed under vacuum. The crude extract obtained after concentration was weighed and extractive values were determined and reported in Table 3.

Phytochemical analysis

The crude extracts were analyzed for the presence of various phytoconstituents by following standard phytochemical tests (Farnsworth, 1966) and the results were reported in Table 4.

Conclusion

In conclusion, the present study on pharmacognostical

characters of *C. berryi* (Arn) Engl will be providing useful information with regard to its correct identity and help to differentiate from the closely related other species of Commiphora.

The parameters observed and reported will be of use to the future workers in selecting the correct herbal specimen.

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