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Original Article

Macro-microscopy and HPTLC Atlas of Heartwood of *Erythroxylum monogynum* Roxb. (Indian Bastard Sandalwood)

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ABSTRACT

Background: *Erythroxylum monogynum* Roxb. (Fam. Erythroxylaceae) is a tree growing up to 9 m in dry evergreen to deciduous forests, distributed in South India mainly on the Deccan plateau, up to 3000 ft. The wood oil possesses characteristic pleasant odour and a pungent taste, hence adulterated with sandalwood oil in perfumery. Traditionally wood oil is used for webbing eczema; bark and wood used for stomach ailments, as a stimulant, diaphoretic, diuretic, for dyspepsia and also for continuous fever. *E. monogynum* is used as adulterant in the herbal drug market for the heartwood of sandal on account of their morphological and organoleptic resemblances. **Objectives:** A systematic pharmacognostical study of dried stem heartwood of *E. monogynum* has been executed to identify the microscopical and phytochemical features. **Materials and Methods:** Morphology, anatomy, powder microscopy and TLC/HPTLC studies were carried out by standard Pharmacopoeial protocols. **Results:** Surface characters, odour, taste, arrangement of xylem elements in TS, TLS, RLS, fibres, tailed pitted vessels, tyloses, xylem rays, axial parenchyma with brownish content, tracheids, fibre tracheids, prismatic crystal, crystal fibre and oil globules were the unique diagnostic characters observed. HPTLC showed 5, 6 and 11 bands under 254 nm, 366 nm and white light (post derivatisation with vanillin-sulphuric acid) respectively. The denistogram showed 12, 10 and 13 peaks at UV 254 nm, 366 nm and 520 nm after derivatisation. **Conclusion:** The findings of the present study will be helpful in identification of this raw drug as a whole or as a powder.

Keywords: *Devadaru*, Market adulterant, Red cedar, Sandalwood, Wood anatomy, Pharmacognosy.

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INTRODUCTION

Erythroxylum monogynum Roxb. syn. *E. indicum* (DC.) Bedd. (Erythroxylaceae) (Figure 1 A and B) has fragrant heartwood. It is found in dry evergreen to deciduous forests growing up to 9 m high in Southern India mainly on the Deccan plateau up to 3000 ft height. It is commonly known as Bastard sandalwood or Red cedar (Eng.); *Sembulichan, Kattuchandanam, Devadaram, Chemanatti* (Tam.); *Davadaru, Adivi gerenta* (Tel.); *Chembulinga, Vella-devadaram* (Mal.); *Gandh-giri* (Mar.) and *Devadaram* (Kan.).[1-5]

The heartwood oil traditionally obtained by steam distillation (yield 1.15 to 2.56%) is composed of hibaene epoxide, alkaloids and diterpenoids (hydrocarbons, epoxides, mono alcohols, diols and triols). Diterpenoids *viz.* (-)-ent-8(17),13(E)-labdadien-15-ol; (-)-pimaradiene (6%); (+)-allodevadarool; diterpene triol-A; (+)-devadarene (7%); (+)-devadarool; (-)-hydroxydevadarool; (+)-hibaene (74%); (+)-hibaene epoxide; (+)-monogynol; diterpene monools A and B; (+)-hydroxymonogynol; (-)-kauranol; (-)-kaurandiol; (-)-atisirene (5%); (-)-isoatisirene (8%) along with β-sitosterol, erythroxytriols Q and P20[6-23] are reported along with sesquiterpenes bisabolene, cadinene and capric acid.[24]

The heartwood is of high demand and used in house construction, fine arts, agricultural tools, in *Yajnas* (a ritual sacrifice with a specific objective), for wood oil (*Sembulichan tailam*) used in the preparation of balm and for treatment of webbing

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Figure 1: *E. monogynum*, A: Habit, B: Flower and fruiting twig.

eczema.[25] The bark and wood are used for stomach ailments, as a stimulant, diaphoretic, diuretic, for dyspepsia and for continuous fever.[26] Reddish brown essential oil is used for timber preservation purposes.[27] The wood oil has characteristic pleasant odour and a pungent taste; it is used as a substitute and adulterant for sandalwood oil and used in perfumery.[28-30] Most often in temple street shops *E. monogynum* heartwood is sold as sandalwood and people buy it based on its aroma and texture.

E. monogynum is locally called as *Devadari, Davadaru, Davadaram* and *Vella-devadaram*. In many Indian languages *Cedrus deodara* (Roxb. ex Lamb.) G.Don. is called as Deodar (Eng.); *Devdaroo, Devadaru* (Hin., Ben. and Tam.); *Devdar* (Guj. and Mar.); *Devtaram* (Mal.); *Deevdar* (Kan.) and *Devdari* (Tel.).[31] Because of these facts this wood is used as an adulterant which causes confusion in local dialect names in India, hence heartwood oil and heartwood cut pieces actively sold as sandalwood and *Davadaru* wood (*Cedrus deodara*) in herbal crude drug markets.[29,32] To detect the botanical identity of the heart wood of *E. monogynum*, cost effective and simple standardization tests such as morphology, anatomy, powder microscopy and TLC/HPTLC were performed and results reported in this paper.

MATERIALS AND METHODS

Authentic stem heartwood of *E. monogynum* was collected from Jawadhu hills at Vellore district, Tamil Nadu, India (12°35′N 78°50′E). The voucher specimen (C/Wd13) was deposited in the museum of department of Pharmacognosy, CSMCARI (CCRAS), Arumbakkam, Chennai, India for future reference.

Macro-microscopy

The heartwood are submerged in water for two days and free hand sections were taken following standard procedures.[33] Heartwood was polished with silicon carbide waterproof paper 320 grit and scanned with canoscan LiDE 300 (3500dpi) and micro-morphological characters were examined under Zeiss Axiolab 5 trinocular microscope fitted with Axiocam 208 color camera. Powder characters were drawn under 200X magnifications with the help of Olympus BX43 trinocular microscope having drawing tube.

Chemicals, Solvents and Reagents

All the chemicals and solvents used were AR grade (Merck). For visualizing the developed spots in TLC, reagent containing vanillin (1 g) sulphuric acid (5%) in ethanol (VSA) was used. The mobile phases used were toluene: ethyl acetate: formic acid (7:2.5:0.5, *v/v/v*) for ethanol extract. This mobile phase offered a good separation of phytoconstituents while running the trial TLCs. Similarly, chloroform: methanol (6:2, *v/v*) lifted the alkaloids in the alkaloid fraction. For HPTLC, aluminium plate (Merck) pre-coated with Silica gel 60 F_{254} of 0.2 mm thickness was used.[34-36]

Instrument for HPTLC

Automatic sampler ATS4 was used for application of extracts on TLC plate; twin trough chamber (10×10 cm) was used for plate development; visualizer was used for photo documentation under UV-Visible conditions; Scanner 4 with winCATS software was used for obtaining densitograms; TLC plate heater was used for derivatization (all from CAMAG, Switzerland).

HPTLC Procedure

Powdered stem heartwood (1 g) was soaked overnight in ethanol (25 ml). Boiled over water bath, filtered and concentrated to 10 ml. For alkaloid fraction preparation, the drug (5 g) was soaked overnight with 5% acetic acid. Then filtered, neutralized with sodium hydroxide and pH was adjusted to 8. Then repeatedly extracted with chloroform, concentrated to 10 ml. Ethanol extract and alkaloid fraction (10 µl on each track) were applied as 8 mm bands on silica gel 60F_{254} coated aluminium plate (8x10 cm) using ATS4 applicator from 10 mm from left side and 10 mm from bottom of the plate. The plate was developed in the mobile phases after pre-saturation of the twin trough chamber (10×10 cm) with mobile phases. The plates were developed up to 90 mm from the bottom. The developed plates were air dried, viewed under 254 nm, 366 nm and the images were documented using visualizer followed by dual wavelength scanning with the aid of Scanner 4 at λ 254 nm (D_2 lamp, absorption mode) and λ 366 nm (Hg lamp, fluorescence mode) with a slit dimension of 6×0.45 mm and scanning speed of 20 mm/s. One plate was dipped in a dip tank containing VSA reagent and heated at 100°C or till the appearance of coloured spots, the derivatized TLC plates were photo documented under white light followed by scanning at λ 520 (W lamp, absorption mode) and other plate was derivatized in Dragendroff's reagent to detect alkaloids.

RESULTS

Macroscopy

Heart wood is very hard and heavy, difficult to break; longitudinally cut pieces are 10 to 15 cm long, 1 to 4 cm wide and 1 to 2 cm thick; surface smooth, fracture sharp and splintery, cut surface shows dark narrow line of late wood and light coloured wide zone of early wood (annular rings), dark brown in color but later on turns slightly reddish-brown (Figure 2 and 3); specific gravity 0.83; odour pleasant, taste slightly bitter and astringent.

Microscopy

TS of heartwood shows simple perforation; diffused porous vessels are mostly arranged in radial multiplication groups of as many as 6 to 8 vessels, minimum 2 and maximum 11 celled group; few are isolated, circular or elliptical in outline, 95 to 120 per mm^2, vessel length 270 to 1200 µm, width 40 to 90 µm, few are up to 120 µm; vessels contain non-lignified thin-walled tyloses with reddish brown content, crystal like dry gum substance and oil globules; xylem rays are storied, filled with brownish content and starch grains; mostly uni and bi-seriate, rarely tri-seriate, running almost straight and parallel, except near to vessels it get slightly bent, inter ray distance 30 to 150 µm; axial parenchyma partially vesicentric (paratracheal) embedded with reddish brown content, gum like substances and oil globules; isolated and irregularly running 3 to 6 cells of metatracheal parenchyma connects adjacent tracheids; fibres, vessels/vessel groups also present the growth ring margin, a few isolated metatracheal parenchyma are embedded with prismatic crystals of calcium oxalate; ground tissue consist non septate, radially arranged, thick walled, narrow lumened fibres which are 600 to 1600 µm in length, 7 to 11.5 µm thick wall and up to 27 µm wide, mostly angular to rectangular and few are oval in shape (Figure 4).

RLS shows heterogeneous xylem rays composed of linear rectangular shaped body ray cells (procumbent), mostly 2 to 14 cells rows having single rows of square shaped marginal

Figure 2: Dried stem heartwood.

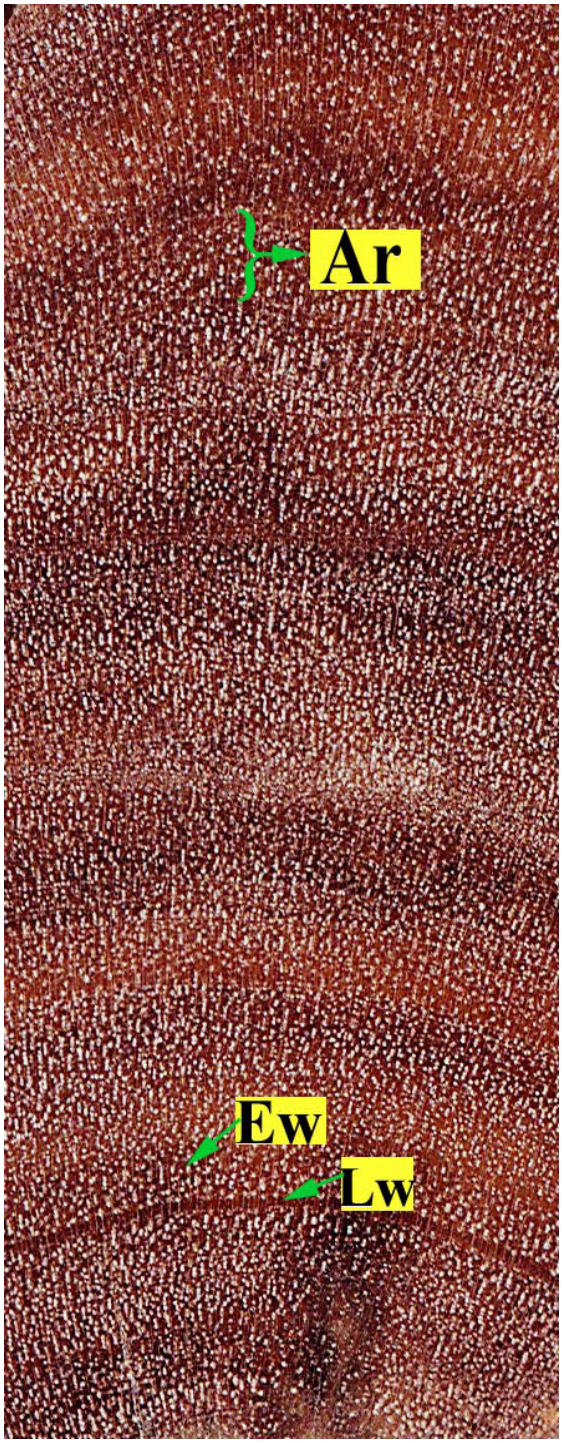


Figure 3: Macro-morphology of transversely cut heartwood.

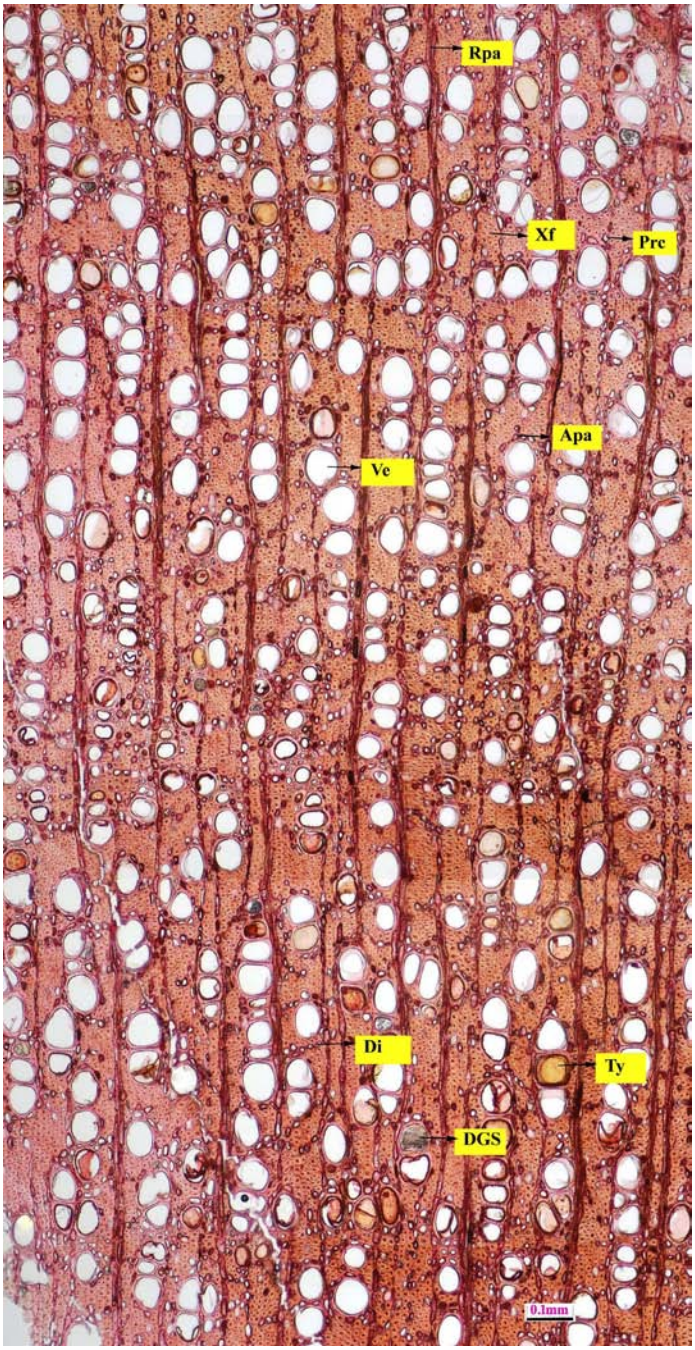


Figure 4: Detailed transverse section of *E. monogynum* stem heartwood.

(upright) cells on both side; thick walled tailed pitted vessels have tyloses with yellow to reddish brown content and white colour dry gummy (crystal mass like, but not glittering under polarizer light) substances; diffused to diffused in aggregate paratracheal and irregularly storied metatracheal axial parenchyma have two distinct sizes with circular and elliptical pits; square shaped metatracheal cells arranged in vertical columns of 10 to 28 cells, maximum 36 cells high, embedded with prismatic crystals of calcium oxalate, rectangular shaped metatracheal cells contain reddish brown content, gum like substances and oil globules (Figure 5 A and B).

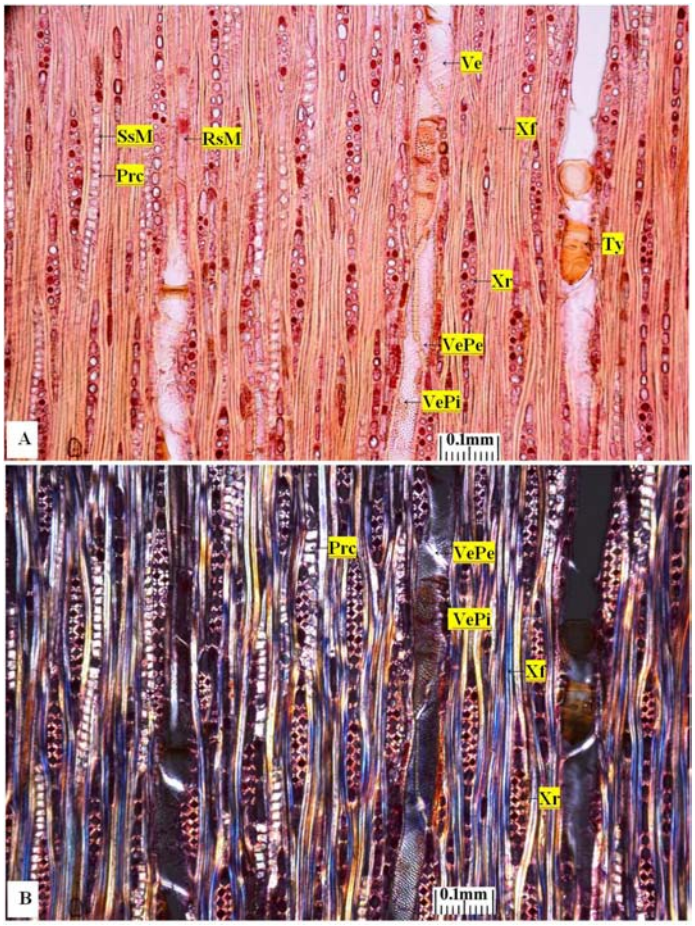


Figure 5: A. Detailed TLS, B. Detailed TLS under polarizer light.

TLS shows thick-walled tailed pitted vessel, inter pits are simple round to oval and at few places elliptical; lenticular shaped ray cells 120 to 350 µm high and 15 to 35 µm wide; rays groups are 38 to 48 per mm^2, uni and bi-seriate, mostly 4 to 16 cell rows high, storied, embedded with reddish brown content, starch grains, oil globules and adjacent to arranged axial parenchyma, pitted vessels with tyloses, fibre-tracheids, fibres (Figure 6 A and B).

Powder Microscopy

Fibres are thick walled, narrow lumened, crossing with thin line like middle lamellae between adjacent walls, mostly non storied, few are embedded with brownish content, ends pointed, length mostly 0.6 to 1.2 mm and few are up to 1.8 mm, width 9 to 40 µm, cell-wall thickness 4 to 19 µm; simple perforated, cylindrical with circular or elliptical opening tailed vessels, tails on both side and few with tails on single side but a little projection is there on the other side, end wall slightly oblique, inter-vessel pits are simple and opposite, mostly oval to circular, vessel ray pits have two distinct sizes, similar to inter-vessel pits and same ray cell 2 to 5 µm in width and elliptical, pits up to 19 µm, vessels have two type of tyloses; prismatic crystal fibre; fibre tracheids having thick-walled simple pits, length 600 to 1050 µm and width 30 to 60 µm; fragment of xylem ray parenchyma having reddish to yellowish brown content embedded with starch grains;

radial longitudinally cut xylem ray crossing with fibre and fibre tracheids; tangential longitudinally cut xylem ray associated with fibre and axial parenchyma; different shape and size xylem parenchyma with oil globule, simple round to oval starch grains of 10 to 70 µm diameter and prismatic crystals of calcium oxalate of 25 to 35 µm across (Figure 7).

HPTLC fingerprint of Ethanol extract of *E. monogynum*

The TLC profile of ethanolic extracts of stem heartwood of *E. monogynum* was developed in the solvent system toluene: ethyl acetate: formic acid (7:2.5:0.5 v/v/v). The solvent system ratio is chosen by trial and error method to obtain distinguishable band separation. TLC finger print showed numerous phytochemicals appeared as bands at 254 nm, 366 nm and vanillin-sulphuric acid reagent, showed 5 bands at 254 nm, 6 bands at 366 nm and plate derivatized with vanillin-sulphuric acid showed 11 bands (Table 1).

The HPTLC finger print profile of ethanol extract of the stem heartwood *E. monogynum* showed 12 spots at UV 254 nm with 2 spots appeared major with area of more than 10% the R_f values (Figure 8); 10 spots with one spots appeared major with an area of more than 10% the R_f values shown at UV 366 nm (Figure 9); after derivatization with VSA the plate scanned at 520 nm showed 13 spots with five spots appeared major with an area of more than 10% (Figure 10). The alkaloid fraction shows two orange spots at R_f 0.35 and 0.85 presence of alkaloid (Figure 11).

DISCUSSION

Incorrect identification of plants species has been resulting in adulteration and substitution of raw drugs in herbal drug industry.[37] Drug adulteration has to be developed as a major area of study under herbal drug standardization and studies on adulteration practices have to be taken up along with identification of the crude drugs. Plant anatomy is an important basic tool for authentication of dried heartwood in various levels (chemically processed, burnt, etc). DNA barcoding is a powerful supplement tool for identification heartwood but due to presence of tannins, resins, gums, essential oils, pigments etc. poses challenges.[38] Microscopic characters could be significantly used for authentication at various levels. Though researchers feels anatomical features are difficult to differentiate in close genera in certain family, thorough analysis at cellular level or powder

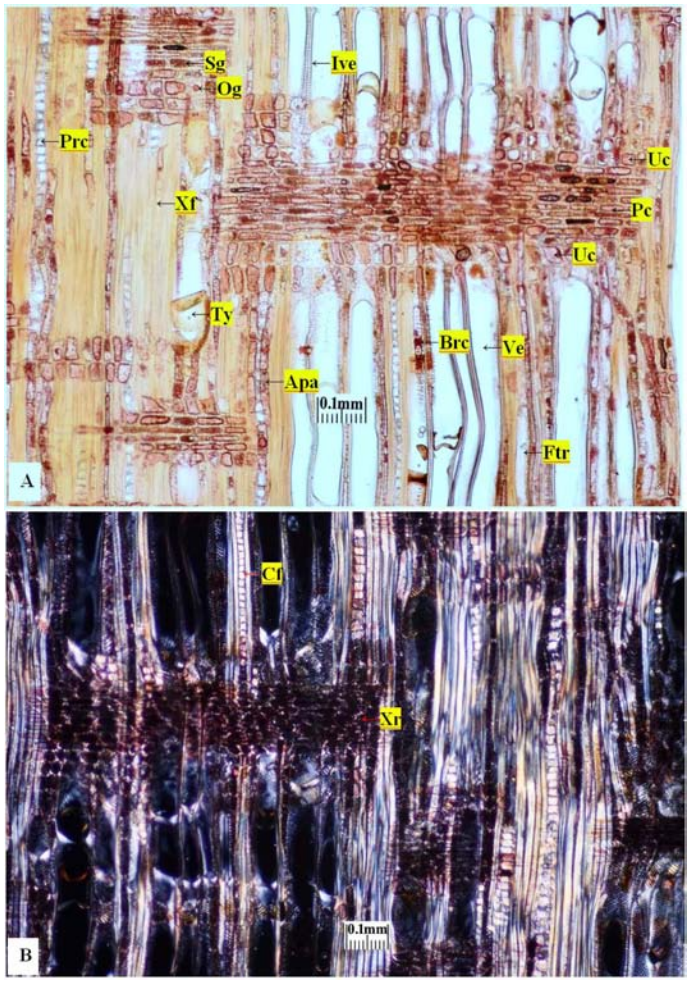


Figure 6: A. Detailed RLS, B. Detailed RLS under polarizer light.

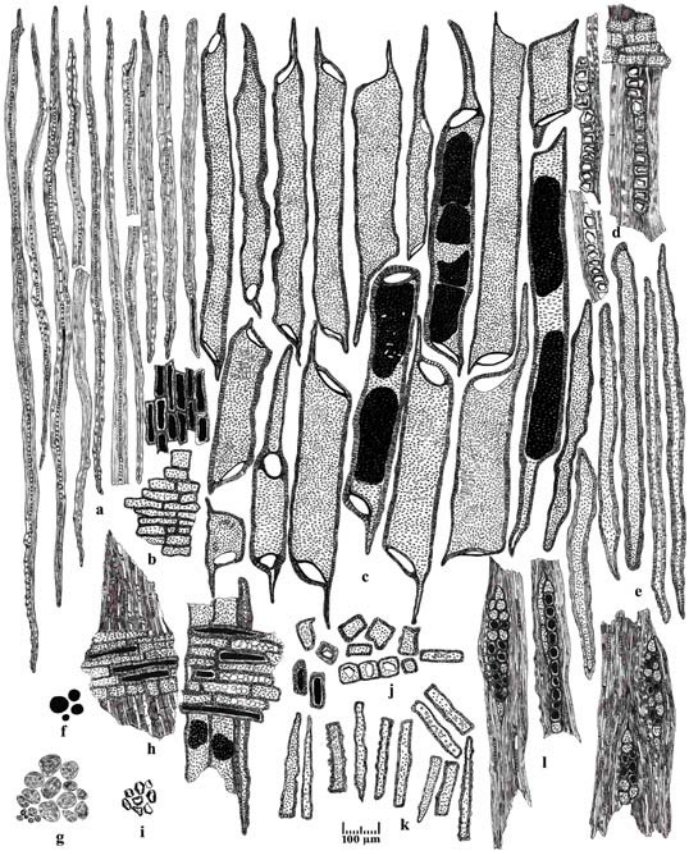


Figure 7: Powder microscopy of *E. monogynum*. a: fibres, b: fragment of xylem ray parenchyma, c: tailed pitted vessels, d: crystal fibre; e: fibre tracheids, f: oil globule, g: starch grains, h: radial longitudinally cut xylem ray crossing with vessels, fibres and fibre tracheids, i: prismatic crystals of calcium oxalate, j: different shape and size of xylem parenchyma cells, k: tracheids, l: tangential longitudinally cut xylem ray associated with fibre and axial parenchyma.

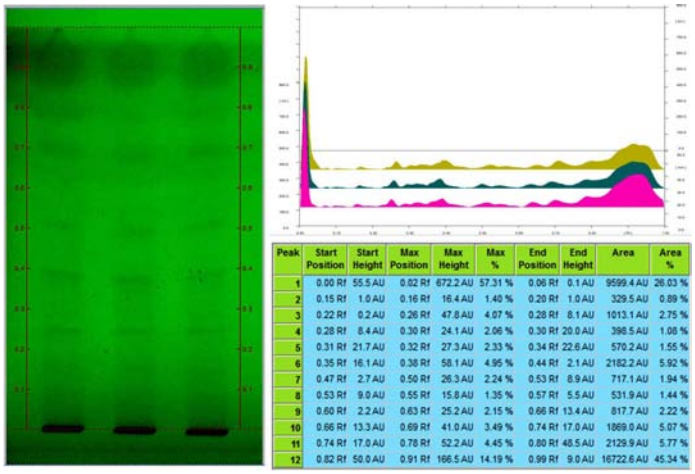
Table 1: R_f values of TLC fingerprint profiling ethanol extract of heartwood of *E. monogynum*

λ=254 nm		λ=366 nm		λ=520 nm (Derivitized)	
R_f	Colour	R_f	Colour	R_f	Colour
-	-	0.03	F Green	-	-
-	-	0.07	F Blue	-	-
-	-	-	-	0.17	Blue
0.25	Green	-	-	-	-
-	-	-	-	0.28	Violet
-	-	0.36	F Blue	-	-
-	-	-	-	0.37	Violet
0.39	Green	-	-	-	-
-	-	-	-	0.45	Pink
0.52	Green	-	-	0.52	Pink
-	-	-	-	0.58	Blue
-	-	-	-	0.65	Pink
-	-	0.68	F Blue	-	-
0.70	Green	-	-	0.70	Violet
-	-	0.74	F Green	-	-
-	-	-	-	0.78	Violet
-	-	-	-	0.84	Pink
0.93	Green	-	-	-	-
--	-	0.97	F Blue	0.97	Violet

microscopy might help further down solving the authentication issues.[40,41]

The vernacular and scientific names differ for *E. monogynum, S. album* and *C. deodara*, but, in the raw drug markets *E. monogynum* heartwood and oil are traded as *S. album* and *C. deodara* in the name of sandal wood, *Davadaram* wood and sandal wood oil.[31] It indicates that the availability of authentic wood for crude drug markets is dwindling or it is not available at all. Some of the researches published on the authentic plant has covered the anatomical and DNA barcoding, but not about the adulterations practices in these wood and powder microscopy work.[8,29,30]

In the present study on stem heartwood external morphology and internal anatomical characters TS, TLS RLS and powder microscopy characters are documented. Quantitative and qualitative macro-micro-morphological characters of stem heartwood can strengthen the taxonomic decisions within the market adulteration. TLC/HPTLC studies are crucial for identification of any herbal drug in addition to microscopic identification. Pharmacopoeias on herbal drugs emphasis the use of TLC for the identification of raw drugs procured from market before using for formulations. The research work to resolve the adulteration available in the herbal market. For the HPTLC, ethanol was used for extracting the phytoconstituents as it is the high polar solvent capable of extracting high polar compounds like glycosides, saponins, tannins, etc. However, for alkaloids

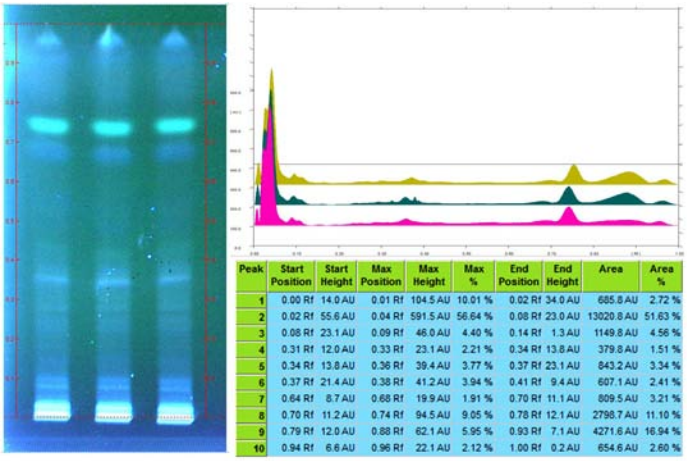


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	55.5 AU	0.02 Rf	672.2 AU	57.31 %	0.06 Rf	0.1 AU	9599.4 AU	26.03 %
2	0.15 Rf	1.0 AU	0.16 Rf	16.4 AU	1.40 %	0.20 Rf	1.0 AU	329.5 AU	0.89 %
3	0.22 Rf	0.2 AU	0.26 Rf	47.8 AU	4.07 %	0.28 Rf	8.1 AU	1013.1 AU	2.75 %
4	0.28 Rf	8.4 AU	0.30 Rf	24.1 AU	2.06 %	0.30 Rf	20.0 AU	398.5 AU	1.08 %
5	0.31 Rf	21.7 AU	0.32 Rf	27.3 AU	2.33 %	0.34 Rf	22.6 AU	570.2 AU	1.55 %
6	0.35 Rf	16.1 AU	0.38 Rf	58.1 AU	4.95 %	0.44 Rf	2.1 AU	2182.2 AU	5.92 %
7	0.47 Rf	2.7 AU	0.50 Rf	26.3 AU	2.24 %	0.53 Rf	8.9 AU	717.1 AU	1.94 %
8	0.53 Rf	9.0 AU	0.55 Rf	15.8 AU	1.35 %	0.57 Rf	5.5 AU	531.9 AU	1.44 %
9	0.60 Rf	2.2 AU	0.63 Rf	25.2 AU	2.15 %	0.66 Rf	13.4 AU	817.7 AU	2.22 %
10	0.66 Rf	13.3 AU	0.69 Rf	41.0 AU	3.49 %	0.74 Rf	17.0 AU	1869.0 AU	5.07 %
11	0.74 Rf	17.0 AU	0.78 Rf	52.2 AU	4.45 %	0.80 Rf	48.5 AU	2129.9 AU	5.77 %
12	0.82 Rf	50.0 AU	0.91 Rf	166.5 AU	14.19 %	0.99 Rf	9.0 AU	16722.6 AU	45.34 %

Figure 8: TLC plate and HPTLC densitogram at 254 nm.

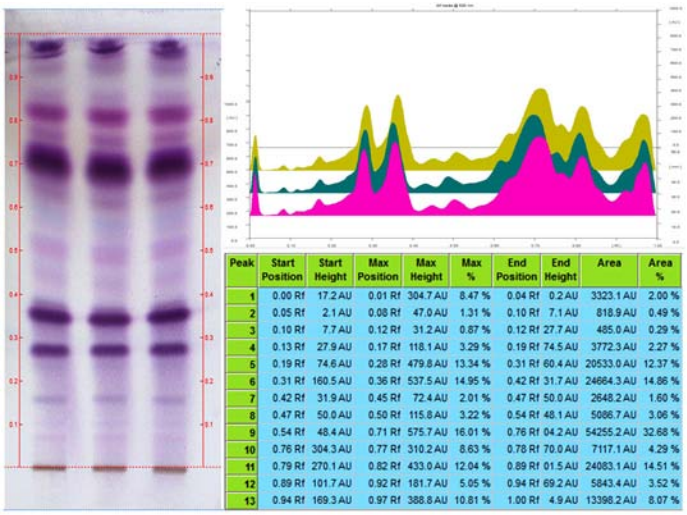
which are present in the form of salts, the alkaloids are extracted by soaking in the acidified aqueous solution, neutralization with a base and extracting with an organic solvent which is immiscible with water.[42]

This study will be helpful for differentiating other controversial sources of *C. deodara* and sandalwood; the *C. deodara* wood is non-porous and presence of fibre tracheids with bordered pits is one of the main distinguishing characteristics.[39] Sandalwood can be anatomically differentiated by the presence of vessels arrangement mostly solitary diffused porous, even 2 or 3 together



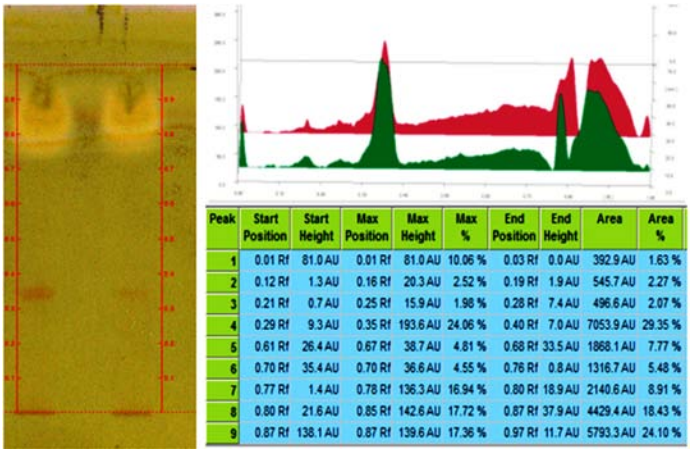
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	14.0 AU	0.01 Rf	104.5 AU	10.01 %	0.02 Rf	34.0 AU	685.8 AU	2.72 %
2	0.02 Rf	55.6 AU	0.04 Rf	591.5 AU	56.64 %	0.08 Rf	23.0 AU	13020.8 AU	51.63 %
3	0.08 Rf	23.1 AU	0.09 Rf	46.0 AU	4.40 %	0.14 Rf	1.3 AU	1149.8 AU	4.56 %
4	0.31 Rf	12.0 AU	0.33 Rf	23.1 AU	2.21 %	0.34 Rf	13.8 AU	379.8 AU	1.51 %
5	0.34 Rf	13.8 AU	0.36 Rf	39.4 AU	3.77 %	0.37 Rf	23.1 AU	843.2 AU	3.34 %
6	0.37 Rf	21.4 AU	0.38 Rf	41.2 AU	3.94 %	0.41 Rf	9.4 AU	607.1 AU	2.41 %
7	0.64 Rf	8.7 AU	0.68 Rf	19.9 AU	1.91 %	0.70 Rf	11.1 AU	809.5 AU	3.21 %
8	0.70 Rf	11.2 AU	0.74 Rf	94.5 AU	9.05 %	0.78 Rf	12.1 AU	2798.7 AU	11.10 %
9	0.79 Rf	12.0 AU	0.88 Rf	62.1 AU	5.95 %	0.93 Rf	7.1 AU	4271.6 AU	16.94 %
10	0.94 Rf	6.6 AU	0.96 Rf	22.1 AU	2.12 %	1.00 Rf	0.2 AU	654.6 AU	2.60 %

Figure 9: TLC plate and HPTLC densitogram at 366 nm.



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	17.2 AU	0.01 Rf	304.7 AU	8.47 %	0.04 Rf	0.2 AU	3323.1 AU	2.00 %
2	0.05 Rf	2.1 AU	0.08 Rf	47.0 AU	1.31 %	0.10 Rf	7.1 AU	818.9 AU	0.49 %
3	0.10 Rf	7.7 AU	0.12 Rf	31.2 AU	0.87 %	0.12 Rf	27.7 AU	485.0 AU	0.29 %
4	0.13 Rf	27.9 AU	0.17 Rf	118.1 AU	3.29 %	0.19 Rf	74.5 AU	3772.3 AU	2.27 %
5	0.19 Rf	74.6 AU	0.28 Rf	479.8 AU	13.34 %	0.31 Rf	60.4 AU	20533.0 AU	12.37 %
6	0.31 Rf	160.5 AU	0.36 Rf	537.5 AU	14.95 %	0.42 Rf	31.7 AU	24664.3 AU	14.86 %
7	0.42 Rf	31.9 AU	0.45 Rf	72.4 AU	2.01 %	0.47 Rf	50.0 AU	2648.2 AU	1.60 %
8	0.47 Rf	50.0 AU	0.50 Rf	115.8 AU	3.22 %	0.54 Rf	48.1 AU	5086.7 AU	3.06 %
9	0.54 Rf	48.4 AU	0.71 Rf	575.7 AU	16.01 %	0.76 Rf	04.2 AU	54255.2 AU	32.68 %
10	0.76 Rf	304.3 AU	0.77 Rf	310.2 AU	8.63 %	0.78 Rf	70.0 AU	7117.1 AU	4.29 %
11	0.79 Rf	270.1 AU	0.82 Rf	433.0 AU	12.04 %	0.89 Rf	01.5 AU	24083.1 AU	14.51 %
12	0.89 Rf	101.7 AU	0.92 Rf	181.7 AU	5.05 %	0.94 Rf	69.2 AU	5843.4 AU	3.52 %
13	0.94 Rf	169.3 AU	0.97 Rf	388.8 AU	10.81 %	1.00 Rf	4.9 AU	13398.2 AU	8.07 %

Figure 10: TLC plate and HPTLC densitogram at 520 nm (Derivatized with VSR).



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	81.0 AU	0.01 Rf	81.0 AU	10.06 %	0.03 Rf	0.0 AU	392.9 AU	1.63 %
2	0.12 Rf	1.3 AU	0.16 Rf	20.3 AU	2.52 %	0.19 Rf	1.9 AU	545.7 AU	2.27 %
3	0.21 Rf	0.7 AU	0.25 Rf	15.9 AU	1.98 %	0.28 Rf	7.4 AU	496.6 AU	2.07 %
4	0.29 Rf	9.3 AU	0.35 Rf	193.6 AU	24.06 %	0.40 Rf	7.0 AU	7053.9 AU	29.35 %
5	0.61 Rf	26.4 AU	0.67 Rf	38.7 AU	4.81 %	0.68 Rf	33.5 AU	1868.1 AU	7.77 %
6	0.70 Rf	35.4 AU	0.70 Rf	36.6 AU	4.55 %	0.76 Rf	0.8 AU	1316.7 AU	5.48 %
7	0.77 Rf	1.4 AU	0.78 Rf	136.3 AU	16.94 %	0.80 Rf	18.9 AU	2140.6 AU	8.91 %
8	0.80 Rf	21.6 AU	0.85 Rf	142.6 AU	17.72 %	0.87 Rf	37.9 AU	4429.4 AU	18.43 %
9	0.87 Rf	138.1 AU	0.87 Rf	139.6 AU	17.36 %	0.97 Rf	11.7 AU	5793.3 AU	24.10 %

Figure 11: TLC plate and HPTLC densitogram of alkaloid fraction (Derivatized with Dragendroff's reagent) at 520 nm.

occasionally, vessel number of 27 to 61 per sq mm, length of 150 to 380 µm, medullar rays are mostly uni and bi-seriate, 6 to 10 ray groups per sq mm, height 160 to 360 µm, 8 to 36 µm wide and 12 to 48 cell rows high; fibre 16 to 20 in diameter, up to 1300 µm in long, stored with brownish content.[28,29] *E. monogynum* has diffused porous vessels arranged in radial multiplication groups of as many as 6 to 8 vessels, but minimum 2 and maximum 11 celled group, 95 to 120 per mm^2, length 270 to 1200 µm, ray cells uni and bi-seriate, 38 to 48 ray groups per mm^2, 120 to 350 µm height, 15 to 35 µm wide and 4 to 16 cell rows high; fibres lumen non-storied, middle lamellae present between adjacent walls, 600 to 1600 µm in length, 7 to 11.5 µm wall thickness and up to 27 in width.

CONCLUSION

This study sets specific macro-microscopic protocol on stem heartwood of *E. monogynum* and also to differentiate it from its substitute and adulterant. The difference in TLC and HPTLC spot in the ethanol extract which can be used for the identification and differentiate the authentic heartwood from the adulterant available in crude drug market will be helpful in differentiating their identity purity self-discipline and also focus on quality control standardization of the herbal drug.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

Apa: Axial parenchyma; **Ar:** Annual ring; **Brc:** Brownish content; **Cf:** Crystal fibre; **Di:** Discontinuous line; **DSG:** Dry gum substance; **Ew:** Earlywood; **F:** Florescent; **Ftr:** Fibre tracheid; **HPTLC:** High-performance thin-layer chromatography; **Ive:** Inter vessel pits; **Lw:** Latewood; **Og:** Oil globule; **Pc:** Procumbent cells; **Prc:** Prismatic crystals of calcium oxalate; **Rpa:** Ray parenchyma; **RsM:** Rectangular shaped metatrachea; **Sg:** Starch grains; **SsM:** Square shaped metatrachea; **TLC:** Thin layer chromatography; **Ty:** Tyloses; **Uc:** Upright cells; **Ve:** Vessel; **VePe:** Vessel perforation; **VePi:** Vessel pits; **VSR:** Vanillin-sulphuric acid; **Xf:** Xylem fibre; **Xr:** Xylem ray.

SUMMARY

This study sets specific macro-microscopic protocol on stem heartwood of *E. monogynum* and also to differentiate it from its substitute and adulterant. The TS, TLS, RLS tissue systems, different shape and sizes of fibres, tailed pitted vessels, tyloses, xylem rays, axial parenchyma with brownish content, tracheids, fibre tracheids, prismatic crystal, crystal fibre and oil globules of powder microscopic characters were studied thoroughly. The difference in TLC and HPTLC spot which can be used for the identification and differentiate the authentic heartwood from the adulterant available in crude drug market will be helpful in

differentiating their identity purity self discipline and also focus on quality control standardization of the herbal drug.

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