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MACROSCOPICAL, MICROSCOPICAL AND PHYSICO-CHEMICAL STUDIES ON LEAVES OF *DALBERGIA SISSOO* LINN. (FABACEAE)

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ABSTRACT: Objective: To rationalize the macroscopical, anatomical and physico-chemical studies on leaves of plant *Dalbergia sissoo* Linn. (Fabaceae). **Methods:** The crude ethanollic extract of leaves of *Dalbergia sissoo* Linn. (Fabaceae) was using physico-chemical parameters, fluorescence analysis, and preliminary phytochemical investigation (TLC, HPTLC, column chromatography). **Results:** An attempt has been made to highlight this folk herbal medicine through present study which will assist in the identification of fresh as well as dried crude samples of leaves anatomically and physiochemically. TLC fingerprint profiling and fluorescence analysis of powdered leaves were also carried out and the salient qualitative and quantitative parameters are reported. **Conclusions:** The present study will provide referential information for correct identification and help in checking adulteration in market samples used in the preparation of various herbal medicines. The present observation will also be helpful in macroscopical, microscopical and physiochemical studies on leaves of *Dalbergia sissoo* Linn.(Fabaceae).


INTRODUCTION: *Dalbergia sissoo*, commonly known as Indian Rosewood, is a deciduous tree, also known as sisu, sheesham, tahli and Tali. *Dalbergia sissoo* is the state tree of Punjab state (India) and the provincial tree of Punjab province (Pakistan). It is found growing along river banks below 900 metres (3,000 ft) elevation, but can range naturally up to 1,300 m (4,300 ft). It can withstand average annual rainfall up to 2,000 millimetres (79 in) and droughts of 3–4 months. It prefers soils from pure sand and gravel to rich alluvium of river banks.

Shisham can grow in slightly saline soils. Seedlings are intolerant of shade.¹ *Dalbergia sissoo* is a medium to large tree of about 25 meters high with grey yellow trunk, 2-3 meters in diameter.

Leaves: are leathery, pinnately compound, alternate leaflets, petiolated leaf stalk, measures about 15 cm long, each leaflet widest at the base, to 6 cm long with a fine pointed tip.

Flowers: are whitish to pink, fragrant, nearly sessile, and in dense clusters.²

Pods: are oblong, flat, thin, strap-like 4–8 cm long, 1 cm wide and light brown. They contain 1–5 flat bean-shaped seeds 8–10 mm long. It has a long taproot and numerous surface roots which produce suckers. Young shoots are downy and drooping, stems have light brown to dark grey bark up to 2.5 cm (0.98 in) thick, shed in narrow strips, large upper branches support a spreading crown.³

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Seeds: are 6-8 x 4-5 mm, kidney shaped, thin and flat, light brown. The fruit is dry and hard. The sapwood is white to pale brown in colour and the heartwood is golden to dark brown in colour. It develops a long taproot from an early age, and numerous lateral ramifying roots.⁴⁻⁵

Different parts such as roots, bark, wood, leaves and seeds are being used as remedy in many diseases including skin diseases, blood diseases, syphilis, stomach problems, dysentery, nausea, eye and nose disorders, aphrodisiac, expectorant. Leaf extract has been used to treat sore throats, heart problems, dysentery, syphilis, and gonorrhoea. In India and Nepal rural people use *Dalbergia sissoo* leaves to treat animals suffering from non-specific diarrhoea.⁶



FIG. 1: LEAVES OF DALBERGIA SISSOO

Pharmacognostic studies and phytochemical action on leaves of *Dalbergia sissoo* L. have promoted us to undertake the present study. (Table 1)

TABLE 1: ETHNOMEDICAL INFORMATION OF DALBERGIA SISSOO LINN.

Form used	Pharmacological activity	Reference
Extract of aerial part	Showed bronchodilation as well as significant antipyretic, analgesic and estrogen like activities	7
Dried leaves	Antibacterial, antiprotozoal and anti-inflammatory activity	8
Leaf Juice	Used in gonorrhoea	9
Wood paste	Used in Wound, Itches, Abscess and Vomiting	9
Oil	Shows repellent activity against <i>Anopheles stephensi</i> , <i>Aedes aegypti</i> , <i>Culex quinquefasciatus</i> and is also resistant to some wood boring insects	10, 11, 12
Wood and active extract of bark	Ayurvedics: abortifacient, anthelmintic, antipyretic, aperitif, aphrodisiac, expectorant, refrigerant, anal disorders, dysentery, dyspepsia, leucoderma and skin ailments, Yunani: wood useful for blood disorders, scabies, eye and nose disorders, burning sensations, scalding urine, stomach problems, syphilis boils, eruptions, leprosy and nausea.	8, 10

MATERIALS AND METHODS:

Plant Material: The plant material was collected from the local region of Bundelkhand Jhansi in the month of December 2015. The plant was identified by local people of that Bundelkhand region and authenticated by Dr. Gaurav Nigam (Asst. Professor) Department of Botany, Bundelkhand University, Jhansi (U.P.) India. A herbarium specimen of the plant (BU/Bot./Spe./Pha./01-2016/01) was preserved in the Department of Pharmacognosy of our Institute for further reference. The fresh leaves were used for the study of macroscopic and microscopical characters. Whereas collected plants leaves were separated and dried under shade, pulverized by mechanical grinder, passed through 40 mesh sieve and stored in a closed vessel for further use. This coarse powder was used for the determination of ash values, extractive values and preliminary phytochemical investigation as standard methods.

Chemicals: The various chemical are used such as ethanol, chloroform, benzene, n-hexane, ethyl acetate, toluene, formic acid, petroleum ether, sodium & pot. hydroxide, sulphuric acid, hydrochloric acid, nitric acid, glacial acetic acid, iodine silica gel 60-80 mesh and Silica gel 60 F254 precoated Aluminium plates 0.2 mm.

Macroscopy: The observed macroscopical characters of leaves of *Dalbergia sissoo* Linn. were as follows:

- Leaf arrangement- Alternate
- Leaf type- Odd pinnately compound
- Leaf margin- Entire
- Leaflet venation- Pinnate
- Leaflet shape- Elliptic (oval) orbiculate
- Leaf size- 2.57-2.80cm length & 2.19-2.40 mm breadth
- Leaf colour- Green (dark)

- Odour- Characteristic
- Taste - Bitter followed by astringent

Microscopic:

T.S of leaf:

Midrib: Transverse section of midrib shows a flat surface on the adaxial side and convexity on the abaxial side (**Fig. 2 A, B**). The epidermis is made up of single layer of rectangular transversely elongated cells (**Fig. 2 A, B**). Abaxial epidermis is papillose and inner walls are gelatinized. The hypodermal region of adaxial and abaxial epidermis is composed of 2 to 4 rows of collenchymas cells.

A large arc shaped collateral vascular bundle is situated in the centre. Sclerenchyma fibres are present on the adaxial and abaxial side of the vascular bundles.

Leaf is dorsiventral in structure. Adaxial epidermal cells are larger than the abaxial epidermal cells. Hypodermis on the upper side is made up of large rectangular parenchyma cells (**Fig. 2. C**). The palisade tissue is made up of 2 rows of columnar closely packed cells. The spongy tissue is composed of 5 to 7 rows of loosely arranged round parenchyma cells. A small crystalline grains or prisms or rod shaped crystals are seen in the mesophyll tissue. The stomatal index for abaxial

epidermis is 17 to 21 (**Fig. 2.D**); palisade ratio 3 to 4; vein islet number 18 to 22 (**Fig. 2.E**). The smaller veins of the leaf are vertically transcurrent.

Petiole: Transverse section of petiole is circular in outline (**Fig 2.F**). The outer most epidermis is made up of single layer of cells. Most of the cells elongate to form uniseriate trichome. Epidermal cells are papillose. The cortex is broad and composed of round, closely arranged parenchyma cells (**Fig 2.G** and **Fig 2.H**). In the centre 'U' shaped with strongly incurved ends and approximately circular, leaving a small gap on the adaxial side, large, collateral vascular bundle is seen. Vascular bundle is surrounded by sclerenchyma fibres(**Fig 2.I**).

Epidermis in surface view: The adaxial foliar epidermis is made up of polygonal parenchyma cells with straight wall and devoid of stomata. Uniseriate trichomes are noticed (**Fig. 2.J**).

Trichome: Trichomes are numerous, simple, uniseriate with a short basal cell accompanied by an elongated terminal cells with blunt tip (**Fig. 2.J, K**). The abaxial foliar epidermal cells are also polygonal in shape with straight walls but smaller in size. It is perforated by rubiceous stomata or stomata surrounded by a rosette of cells (**Fig. 2. L**).

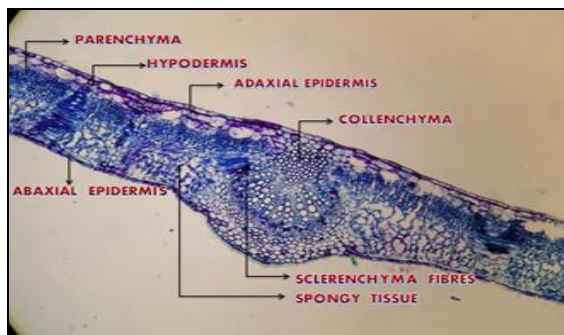


Fig. 2. A: T.S. of leaf

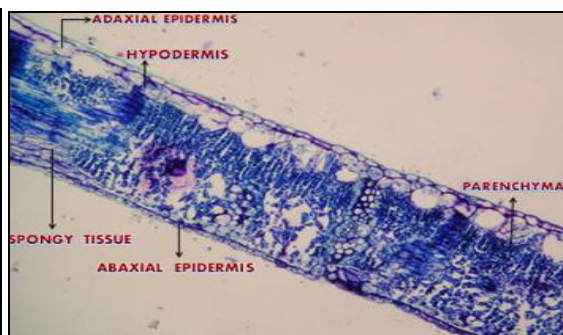


Fig. 2. B: T.S. lamina



Fig. 2. C: Adaxial foliar epidermis



Fig. 2. D: Stomata

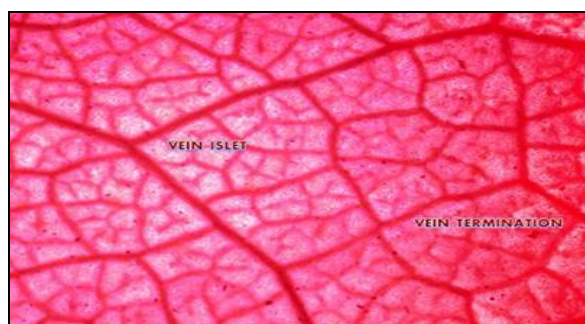


Fig. 2. E: Vein islets



Fig. 2. F: T.S. of petiole

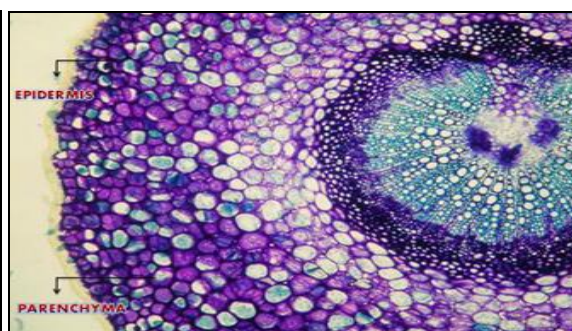


Fig. 2. G: T.S. of petiole – A portion enlarged

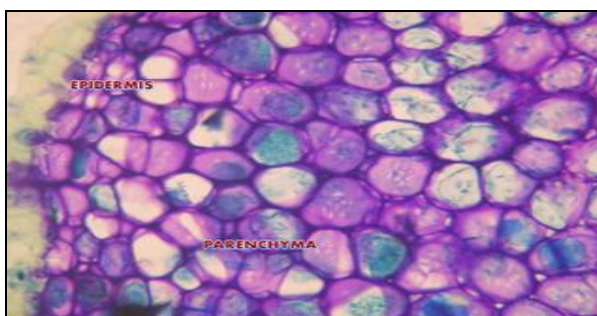


Fig. 2. H: T.S. of petiole showing cortical cells

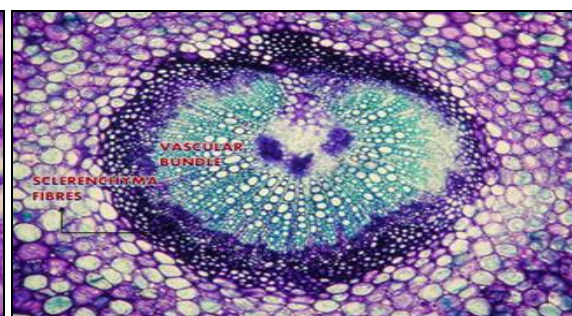


Fig. 2. I: T.S. of petiole Vascular



Fig. 2. J: Trichome – Enlarged



Fig. 2. K: Trichome



Fig 2. L: Stomata enlarged

FIG. 2: MICROSCOPIC OF *DALBERGIA SISSOO* LINN. LEAVES

Extraction of plant materials: A total of 250 g coarse powder of air dried leaves of *Dalbergia sissoo* were packed in muslin cloth and subjected to Soxhlet extractor for continuous hot extraction with petroleum ether and ethanol for 8 h separately. Then the each extracts were filtered and filtrate was evaporated to dryness. The percentage yield of the petroleum ether and ethanol extracts is given below in **Table 2** respectively.

TABLE 2: EXTRACTION OF PLANT MATERIALS

S.no.	Solvent	Wieght of drug (g)	% yield
1	Pt. Ether (80%)	250	3.342
2	Ethanol (100%)	250	8.198

Physico-chemical parameters: Leaves of *Dalbergia sissoo* such as total alcohol soluble extractive, water soluble extractive, ash value, acid insoluble ash, water-soluble ash, loss on drying, are presented in **Table 3**.

TABLE 3: PHYSICAL PARAMETER OF LEAVES OF DALBERGIA SISSOO

S. no.	Parameter	Results(%)
1	Total ash value	8.24
2	Acid insoluble ash value	3.36
3	Water soluble ash value	4.7
4	Water soluble extractive value	9.84
5	Ethanol soluble extractive value	8.198
6	Pt. Ether soluble extractive value	3.342
7	Loss on drying	18.38

Fluorescence analysis: The fluorescence analysis of the powdered drug of *Dalbergia sissoo* in various solvents and chemical reagents was performed under normal and 254 nm and 366 nm UV light. The fluorescence analysis of the powdered drug of *Dalbergia sissoo* Observed the different colour under UV light is given in **Table 4**.

TABLE 4: FLUORESCENCE ANALYSIS OF POWDERED LEAVES OF DALBERGIA SISSOO

S.no.	Solvent	UV light (254 nm)	UV light (366 nm)
1.	Aqu.1M NaOH	Green	Brown
2.	Alcoholic 1M NaOH	Yellowish green	Light brown
3.	50% H ₂ SO ₄	Green	Dark brown
4.	10% KOH	Yellow green	Dark brown
5.	Iodine	Orange	Brown
6.	50% HCl	Light green	Reddish brown
7.	50% HNO ₃	Light green	Dark brown
8.	Glacial acetic acid	Yellowish green	Light brown
9.	Benzene	Green	Reddish brown
10.	Chloroform	Light green	Blackish brown

Qualitative and Quantitive evaluation parameters: The calculated Quantitative values like stomatal number, stomatal index, vein-islet number, vein termination numer and Palishade ratio, of the leaves of *Dalbergia sissoo* are showed in **Table 5**.

TABLE 5: RESULT OF QUANTITATIVE MICROSCOPY OF DALBERGIA SISSOO LEAVES

S. no.	Parameter	Result
1.	Stomatal number (Upper surface) (Lower surface)	30.65 16.04
2.	Stomatal index (Upper surface) (Lower surface)	20.96 23.86
3.	Vein-islet number	17.78
4.	Vein termination number	5.37
5.	Palishade ratio	3.65

Preliminary photochemical investigation: Photochemical tests were done in plant extracts for the detection of presence of different chemical constituents such as; alkaloids, glycosides, flavonoids, essential oils, carbohydrates, proteins, tannins and other substances which are responsible for the biological activity. So the chemical tests are performed in the ethanolic extract of *Dalbergia sissoo*. For the detection of different chemical constituents are observed in the **Table 6** given below respectively.

TABLE 6: DATA FOR THE PHYTOCHEMICALS SCREENING OF POWDERED LEAVES OF PET. ETHER AND ETHANOLIC EXTRACT OF DALBERGIA SISSOO LINN.

S. no.	Tests	Pt. Ether extract	Ethanolic extract
1	Carbohydrates		
	Molish test	+ve	+ve
	Fehling's test	+ve	+ve
	Benedict's test	+ve	+ve
2	Proteins		
	Biuret test	+ve	+ve
	Millon's test	+ve	+ve
	Precipitation test	+ve	+ve
3	Alkaloids		
	Mayer's test	-ve	-ve
	Hager's test	-ve	-ve
	Wagner's test	-ve	-ve
	Dragendroff's test	-ve	-ve
4	Glycosides		
	Killer-killiani test	-ve	-ve
	Baljet test	-ve	-ve
5	Steroids		
	Salkowski test	+ve	+ve
6	Flavanoids		
	Lead acetate	+ve	+ve
	NaOH solution	+ve	+ve

7	Tannins		
	5% FeCl ₃ solution	-ve	+ve
	Dil. Iodine solution	-ve	+ve
8	Dil. HNO ₃	+ve	+ve
	Saponins		
9	Foam test	-ve	+ve
	Terpenoids		
	Salowski test	+ve	+ve
	Ethyl acetate & Dil.	+ve	+ve
	NH ₃ soln.		
	Fatty acid & oils	-ve	+ve

Thin layer chromatography: “Their relative polarities which related to the type and number of functional groups present on a molecule capable of hydrogen bonding”

$$R_f = \frac{\text{Distance travelled by solute front from origin line}}{\text{Distance travelled by solvent front from origin line}}$$

Where R_f = Retention factor

The ethanolic extract of leaves of *Dalbergia sissoo* Linn was subjected to thin layer chromatography studies, to find the presence of number of compounds which support by the chemical test. R_f value and colour of TLC spots, in solvent system of Toluene: Ethyl acetate: Formic Acid (7:3: few drops). These TLC spots with R_f value and colour are in **Table 7**, and TLC plate in **Fig. 3** is given below.

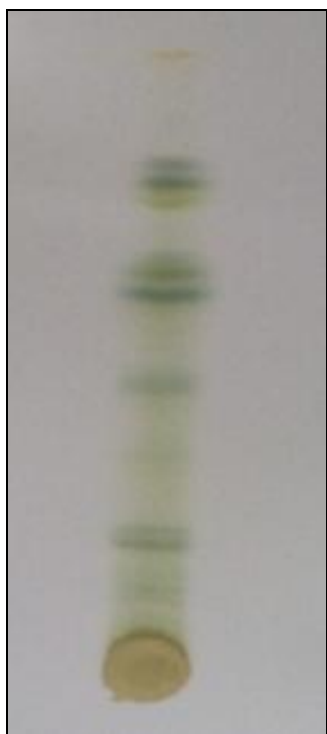


FIG. 3: TLC FINGER PRINTING OF ETHANOLIC EXTRACT ON LEAVES OF DALBERGIA SISSEO

TABLE 7:TLC FINGER PRINTING OF ETHANOLIC EXTRACT OF LEAF OF DALBERGIA SISSEO SPOTS

Extract	Solvent system	No. of spot	Colour of spot	R_f value
Ethanolic extract	Toluene: Ethyl acetate :Formic acid (7:3:few drop)	8	Green	0.750
			green	0.720
			Yellowish green	0.676
			Green	0.617
			Green	0.558
			Yellowish green	0.529
			Light green	0.176
			Light green	0.161

HPTLC finger printing: Ethanolic extract was developed on chromatographic plates with many ratios of different solvents and the best eluent mixture was used further for HPTLC profile to minimize errors in TLC pattern. The preliminary HPTLC studies revealed that the solvent system toluene: ethyl acetate and few drops of formic acid (7:3:few drops) was ideal and gave well resolved sample peaks. **Fig. 4, 5, 6** and **7** HPTLC finger printing of ethanolic extract on leaves of *Dalbergia sissoo* given the spots of the chromatogram were visualized at 254 nm and 366 nm.

Preparation of sample: Dissolved liquid extract in 10 ml ethanol. Filtered using (0.45 μ m) (milipore) filter paper and applied 8 μ l of the solution for Chromatography.

Procedure: High Performance Thin Layer Chromatography (HPTLC) was carried out on Silica gel 60 F254 precoated Aluminium plates 0.2 mm thickness, Merck India Limited Mumbai. An Applicator from Camag Linomat-5 (Camag Switzerland: 140443) was used for band application and photo documentation unit (Camag Reprostar-3: 140604) was used for documentation of chromatographic fingerprints. The mobile phase used Toluene: Ethyl acetate (7:3) solution. The plate ware developed over a distance of 9 cm in a saturated development chamber (Twin through chamber 10X10 cm with SS lid and visualized under as given in each separate sample report. After spraying with 5% methanolic sulphuric acid reagent followed by heating at 105 °C for 5-10 minutes.

TABLE 8: SHOWING R_f VALUES OF HPTLC FINGERPRINTS PROFILE IN TEST SOLUTION OF DALBERGIA SISSOO L. LEAVES

R _f value	At 254nm (Before derivatization)	At 366nm (Before derivatization)
R _f 1	0.34(black)	0.07(orange)
R _f 2	0.66(black)	0.12(orange)
R _f 3	0.93(black)	0.20(light orange)
R _f 4	-	0.35(orange)
R _f 5	-	0.38(purple)
R _f 6	-	0.43(orange)
R _f 7	-	0.47(purple)
R _f 8	-	0.56(pink)
R _f 9	-	0.60(purple)
R _f 10	-	0.65(violet)
R _f 11	-	0.74(orange)
R _f 12	-	0.77(blue)
R _f 13	-	0.80(light orange)
R _f 14	-	0.85(orange)

TABLE 9: SHOWING R_f VALUES OF HPTLC FINGERPRINTS PROFILE IN TEST SOLUTION OF DALBERGIA SISSOO L. LEAVES

R _f values	Under UV light (Afterderivatization)	At 366nm (After derivatization)
R _f 1	0.04(blue)	0.05(orange)
R _f 2	0.31(yellow)	0.11(light blue)
R _f 3	0.90(brown)	0.19(faint blue)
R _f 4	-	0.36(sky-blue)
R _f 5	-	0.47(faint blue)
R _f 6	-	0.60(fluorescent)
R _f 7	-	0.65(blue)
R _f 8	-	0.83(sky-blue)
R _f 9	-	0.91(brick red)

HPTLC Finger prints of test solution of Dalbergia sissoo L. Leave:

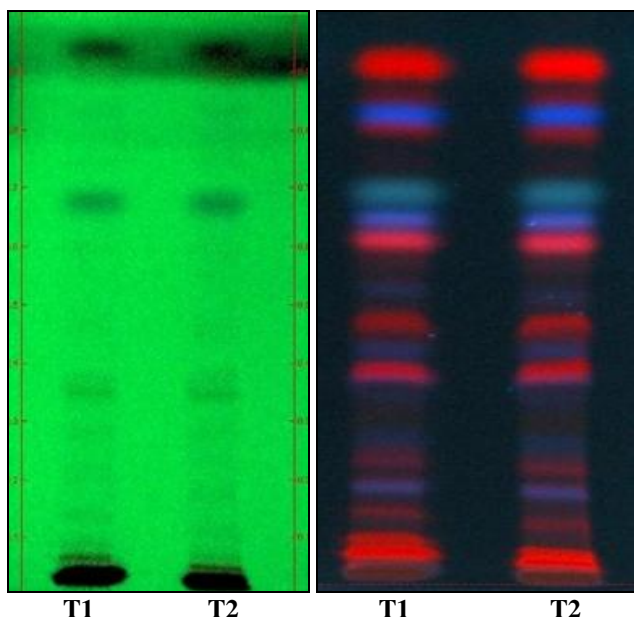


FIG. 4: AT 254 nm (BEFORE DERIVATIZATION) FIG. 5: AT 366 nm (BEFORE DERIVATIZATION)

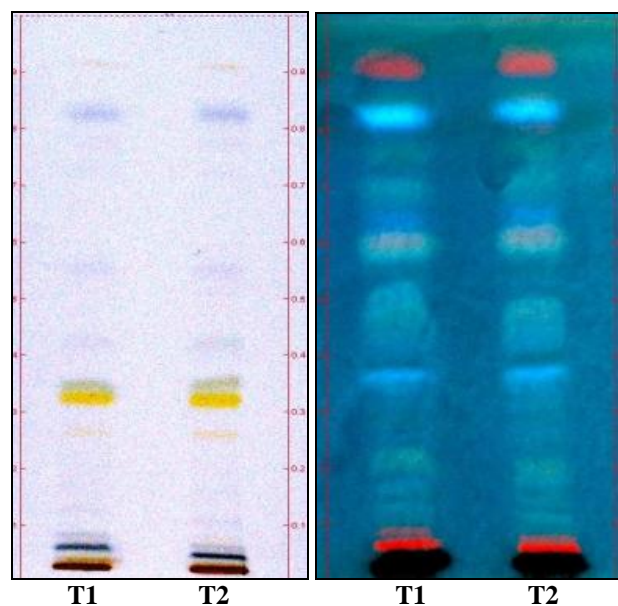


FIG. 6: UNDER UV LIGHT (AFTER DERIVATIZATION) FIG. 7: AT 366nm (AFTER DERIVATIZATION) Where Track T1 and Track T2 (in duplicate)

Column chromatography: The basic principle lying in the column chromatography is adsorption of component at solid-liquid interface. For good separation, the component of mixture should have different degree of affinity for the solid support. The component having strong adsorption for column material is held up while that component having less affinity moves down the column at faster rate as the elute passes through the column.



FIG. 8: COLUMN OF THE ETHANOLIC EXTRACT OF DALBERGIA SISSOO LEAVES

Column chromatography is separated into two categories depending on how the solvent flows down the column. If the solvent is allowed to flow down the column by gravity or percolation, it is called gravity column chromatography. If the solvent is forced down the column by the air

pressure, it is called flash chromatography. Data of column chromatography ethanolic extract of *Dalbergia sissoo* Linn. leaves is given below respectively in **Table 10** and column chromatography in **Fig. 8** is given below.

TABLE 10: DATA OF COLUMN CHROMATOGRAPHY ETHANOLIC EXTRACT OF *DALBERGIA SISSOO* LEAVES

Column fraction no.	Eluent	TLC solvent system	Colour of fraction	No. of spot	R _f value & code
1.	1(1-5)	n-Hexane	100	-	-
2.	2(6-10)	n-Hexane:Toluene	95:5	-	-
3.	2(11-15)	n-Hexane:Toluene	95:5	-	-
4.	3(16-20)	n-Hexane:Toluene	85:15	-	-
5.	3(21-25)	n-Hexane:Toluene	85:15	-	-
6.	4(26-30)	n-Hexane:Toluene	75:25	3	0.75,0.72,0.676
7.	4(31-35)	n-Hexane:Toluene	75:25	3	0.75,0.72,0.676
8.	4(36-40)	n-Hexane:Toluene	75:25	3	0.75,0.72,0.676
9.	5(41-45)	n-Hexane:Toluene	65:35	2	0.72,0.676
10.	6(46-50)	n-Hexane:Toluene	55:45	2	0.72,0.676
11.	7(51-55)	n-Hexane:Toluene	45:55	2	0.72,0.676
12.	8(56-60)	n-Hexane:Toluene	35:65	2	0.72,0.676
13.	9(61-65)	n-Hexane:Toluene	25:75	2	0.72,0.676
14.	10(66-70)	n-Hexane:Toluene	15:85	2	0.72,0.676
15.	11(71-75)	n-Hexane:Toluene	5:95	2	0.72,0.676
16.	12(76-80)	Toluene	100	2	0.72,0.676
17.	13(81-85)	Toluene:Ethyl acetate	95:5	2	0.72,0.676
18.	14(86-90)	Toluene:Ethyl acetate	95:5	2	0.72,0.676
19.	15(91-95)	Toluene:Ethyl acetate	95:5	1	0.676, D ₁
20.	16(95-100)	Toluene:Ethyl acetate	95:5	1	0.676, D ₁
21.	17(101-105)	Toluene:Ethyl acetate	95:5	1	0.676, D ₁
22.	18(106-110)	Toluene:Ethyl acetate	95:5	1	0.676, D ₁
23.	19(111-115)	Toluene:Ethyl acetate	95:5	1	0.676, D ₁
24.	20(116-120)	Toluene:Ethyl acetate	95:5	1	0.676, D ₁
25.	21(121-125)	Toluene:Ethyl acetate	95:5	1	0.676, D ₁

RESULTS: As a part of standardization study, the macroscopically examination of drug was studied. The results showed greater extractive values in hot extraction, indicating the effect of elevated temperature on extraction. Percentages of the extractive values were calculated with reference to air-dried drug. The percent extractives in different solvents indicated the quantity and nature of constituents in the extracts. The extractive values are also helpful in estimation of specific constituents soluble in particular solvent.

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