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Phytochemical Standardization of Oleo Resin of *Shorea robusta* Gaertn f. (Dipterocarpaceae) with modern analytical technique.

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Abstract

More than 70% of India's 1.1 billion populations still use non-allopathic systems of medicine like Ayurveda, Unani, Homeopathy and other systems. The global knowledge about Unani and Indian herbals will hopefully be enhanced by information on the evidence-base of these medicinal plants. Resin of *Shorea robusta* Gaertn known as "Raal" in Unani; Shala in Ayurvedic System belonging to Dipterocarpaceae family plays a foremost role in the Unani system of medicine. It is abundantly available in different parts of Eastern Ghats of Southern Peninsular India and has been widely used in indigenous system for the preparation of different formulations used in the treatment of many skin diseases mainly allergies; diarrhea, dysentery, astringent and as an ingredient in ointments. Due to its therapeutic potentials and vital medicinal properties and also usage of drug in most of the common ailments, lead us to standardize the drug according to WHO guidelines. Phytochemical and physico-chemical studies, macroscopic and microscopic properties of resin were carried out. The main aspects included in the study are organoleptic characters, physico-chemical constants, fluorescence analysis of powdered drug and extracts, TLC profile and Heavy metal analysis, which provide information, which are widely accepted in the quality assessment of herbal drugs and to lay down the standard for the genuine drug. Phytochemical screening, isolation of chemical constituents will help in future to study the pharmacological properties.

Keywords: *Shorea robusta* Gaertn, physico-chemical analysis, TLC, Phytochemical screening.

Introduction

Shorea robusta Gaertn f. (Family-Dipterocarpaceae) is a deciduous tree (Fig 1) widely distributed in India, from Himachal to Orissa eastern districts extending further to the eastern ghats of Andhra Pradesh [1]. It is commonly known as Raal in Unani system of medicine and in English Sal tree, Indian dammer; Shala in Ayurveda has been widely used in indigenous system in the preparation of different formulations for the treatment of many skin diseases mainly allergies; diarrhea, dysentery, astringent, gonorrhea [2] and also used as an ingredient in ointments for skin diseases and ear troubles[3-6] and produced in large quantities in India and constitutes one of the resins of commerce called Sal Dammar or Bengal Dammar (Laldhuna ral, dhup, guggal) (Fig 2).

Fig. 1. *Shorea robusta* Gaertn. f.

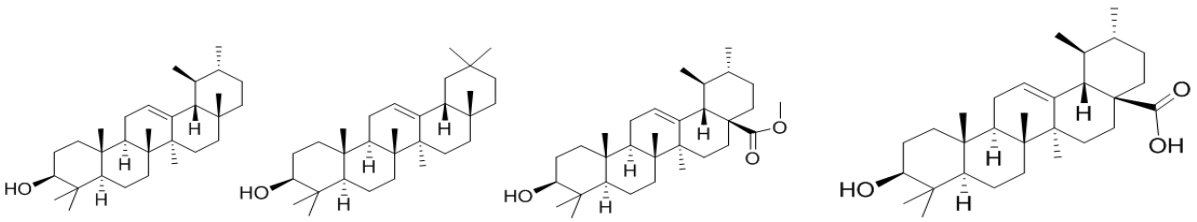
Fig. 2. Macroscopical feature of Oleo resin of *Shorea robusta* Gaertn. f.

It occurs in rough, brittle pieces having a faint resinous, balsamic odour and is widely used as an incense in Indian religious ceremonies as it emits copious white fumes when burnt [7]. A combination of oleoresin with cow ghee is claimed to control burning sensation of haemorrhoids, pain and swelling [8].

Bark is dark brown, seldom quite leafless, smooth or with a few longitudinal cracks. Leaves 10-30cm x 5-18 cm, ovate-oblong, acuminate, tough and shining when mature, petiole 1.2-2cm. long, yellowish, in terminal and Calyx-tube short, not enlarged in fruit [1]. The resin is cooling but difficult to digest; bitter and acrid; astringent to the bowels; purifies the blood; lessens perspiration and fever; good for wounds, ulcers, burns, pains, itching, fractures, useful in dysentery; good for vaginal discharges (Ayurveda). The resin has a bad taste and smell; tonic to the brain; good in ascites, menorrhagia, enlargement of the spleen, obesity, ulcers, and wounds; useful in toothache; as a collyrium good for eyesores and burning of eye.- The oil is good for skin diseases, scabies and all kinds of wounds(Unani). It is used in dysentery, and for plasters and fumigations. It is commonly given for weak digestion, gonorrhoea, and as an aphrodisiac [1]. Useful remedy to take *S. robusta* 4 parts, Mocharas 2, dried decorticated mango kernel 5, *Aegle marmelos* 5 and Nutmeg 5 parts. Mix and make a powder. Dose is 5 grains; used in diarrhea [9].

Chemical constituents

gum resin of *S. robusta* contain ursolic acid and -amyrenone; & -amyrin [5,10]; bark contain ursonic acid and oleanane [11]; seed contain hopeaphenol, leucoanthocyanidin, and 3,7-dihydroxy-8-methoxyflavone7-*O*- -l-rhamnopyranosyl-(1 4)- -l-rhamnopyranosyl-(1 6)- -d-glucopy- ranoside [12]. Structures of chemical constituents were shown in the figure 3.



-amyrin β-amyrin Ursolic acid methyl ester Ursolic acid

Fig. 3. Structures of chemical constituents of Oleo resin of *Shorea robusta* Gaertn. f.

Materials and methods

Oleo resin of *Shorea robusta* Gaertn collected from Talakona, Chittoor District, in January 2011. The sample specimens along with bulk quantities were collected and verified their identification and authentification with the help of botanist and a voucher specimen (No. 3180) deposited in the Herbarium at Central Research Institute of Unani Medicine, Hyderabad.

The present investigation includes parameters such as morphological studies, physico-chemical parameters and HPTLC fingerprint of different solvent extract of oleo resin. Physico-chemical parameters were determined according to the methods described in Anonymous, 2009 [13]. Phytochemical screening was also performed. Fluorescence analysis was carried out as per the method described by Trease and Evans, 1972 [14].

Physico-Chemical parameters of oleo resin of *Shorea robusta* were studied as shown in the table 1 such as total ash, water and alcohol soluble matter, Petroleum ether(60^0-80^0C), Chloroform and alcoholic extractive values, P^H and moisture content (loss on drying at 105^0C), and Phytochemical screening was carried out in the different extracts such as the n-Hexane, Petroleum ether, Chloroform, Ethyl acetate, Ethanol, Methanol, Acetone and

aqueous extract as per the methods described by Evans and Trease, 1972; to know the nature of phyto-constituents present in the drug as shown in the table 3. Phyto-constituents such as terpenoids, flavonoids, saponins and tannins are the major active constituents in the drug as shown in the table 2.

GBC-908 AA model Atomic Absorption Spectrophotometer (AAS) was used to determine heavy metal content as shown in the table 3 and Aflatoxins contamination as shown in the figure 5 were analyzed as per the methods described in WHO guidelines Anonymous, 1998 [15]. Fluorescence analysis of powdered drug and Fluorescence analysis of powdered drug extracts in different solvents were tabulated in table 5 and 6 respectively.

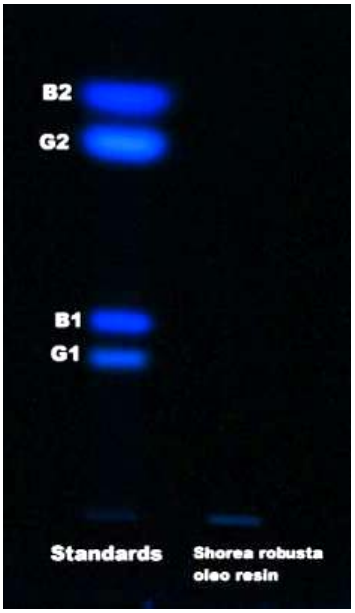


Fig.5 TLC Plate of Aflatoxins standards and *Shorea robusta*.

TLC fingerprint profile

Preparation of extract of the sample drug

Five grams of powdered drug was macerated in 100 ml of Chloroform, Ethyl acetate, Ethanol and Acetone separately in a stoppered conical flask and was kept for 12 hours while shaking at regular intervals. Later the contents were filtered through whattmann No. 41 paper and evaporate the solution to 20 ml. Thus obtained solutions were used as samples for the separation of components.

Development and determination of the solvent system

The samples were spotted as 8mm band on Precoated Aluminium Sheets of Silica Gel 60 F_{254} (Merck) in duplicate as shown in the figure and suitable solvent system as toluene: ethyl acetate: methanol (7:2:1) in the table 7 and developed in the Twin through TLC chamber to the maximum height of the plate so that components are separated on the polar phase of silica gel and mobile phase of solvent system.

Detection system

After the developing, the TLC plate was dried completely and detected under the UV, visible chamber and by spraying with Anisaldehyde Sulphuric acid and heating the plate at 105^0C for 10 minutes and then observed in the UV chamber for detection of spots and photographed at different wavelength as shown in figure 6(a-d). Overlay densitogram for chloroform, ethyl acetate, ethanol and acetone is given using HPTLC system of DESAGA, Sarstedt Gruppe, Germany with automatic applicator as shown in the figure 6e and multiwavelength spectrum for the tlc plate densitogram is given in the figure 6f.

Results and Discussion

Organoleptic Characters

Oleo resin (gum) of *Shorea robusta* Gaertn (Dipterocarpaceae family) is whitish brown in colour; bitter and acrid taste.

Identification

Macroscopy

The resin of *Shorea robusta* has brittle pieces, rough having a faint resinous as shown in the figure 2, whitish brown, freely flowing on the surface of water, odour balsamic, taste acrid

Powder Microscopy

Powder microscopical studies revealed presence of resin with irregular shape, translucent and with reddish tannin contents. Stone cells 10x by 40x and yellow coloured oil globules are also present (Fig 4a-f).

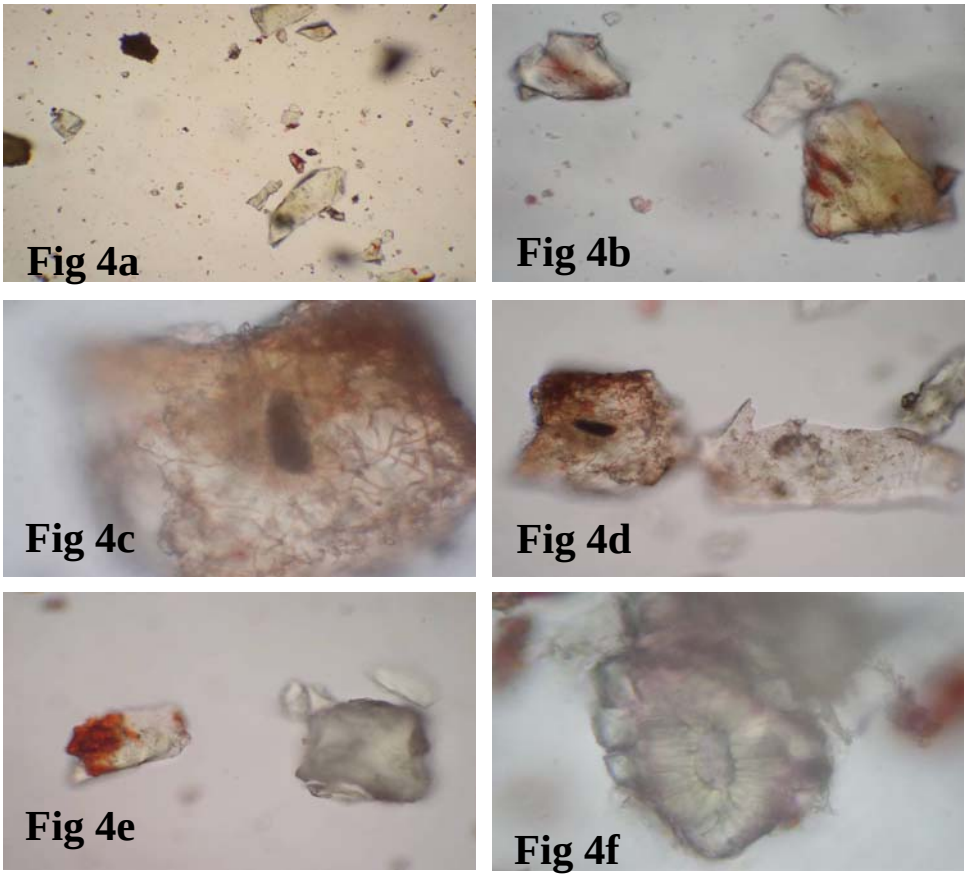


Fig. 4. Powder characters of Oleo resin of *Shorea robusta* Gaertn. f.

Physico-Chemical Standards

Physico-Chemical Parameters data expressed here as mean ±SE. The preliminary phytochemical studies revealed the presence of different phytoconstituent groups in different solvent extracts of the drug. Chloroform, Ethyl acetate, Ethanol and Acetone extract of the drug were carried out TLC analysis whose chromatogram was developed using the solvent toluene: ethyl acetate: Methanol (7:2:1) and detected under the UV 366nm and derivatizing with anisaldehyde sulphuric acid and observed in visible region and at 580nm clearly shown different spots with Rf values as shown in the figure 4. The study carried out on heavy metals such as cadmium, lead, mercury and arsenic were found to be absent as given in table-6. Similarly Aflatoxins were analyzed and found to be absent as given in the table-7 inferring the drug to be safe and non toxic. Powdered drug was screened for fluorescence characteristic with or without chemical treatment. The observations pertaining to their colour in daylight i.e., visible region and under ultra-violet light were noticed and are presented in the table-8. Fluorescence analysis of powdered drug extracts in different solvents was observed and reported in the table-9.

Table.1. Physico-chemical parameters of the oleo resin of *Shorea robusta* Gaertn:

Parameters	Mean ± SE
Total ash (% w/w)	0.054±0.005
Acid insoluble ash (% w/w)	Negligible
Alcohol sol. Matter (%w/w)	56.016±0.010
Water sol. matter (% w/w)	0.1440±0.003
Successive extract (%w/v) Petroleum ether extract Chloroform extract Alcohol extract	 40.380±0.030 87.184±0.020 57.640±0.015
Total moisture content (Loss of weight on drying at 105^0C)	3.968±0.050
P^H of 1% Aqueous Solution P^H of 10% Aqueous Solution	5.89 5.34

Table.2. Phytochemical screening of the nature of compounds present in the drug.

S. No.	Phyto-constituent	Ethanol ext.	Methanol ext.	Chloroform ext.	Pet. ether ext.	Ethyl acetate ext.	Acetone ext.	Aqueous ext.	n-Hexane ext.
1.	Alkaloid	+++	++	++	-	-	+	-	-
2.	Tannins	+	+	+	-	-	+	-	-
3.	Steroids	+	+	+++	-	+	-	-	-
4.	Saponins	+	+	+++	+	+	+	+	+
5.	Flavonoids	+++	+++	+++	++	++	+	+	+
6.	Volatile oil	-	-	+	+	-	-	-	+
7.	Carbohydrates	-	-	++	-	-	+	+	-
8.	Glycosides	+	+	+	-	-	-	-	-
9.	Phenols	+	+	+	-	-	+	+	-
10.	Proteins	+	+	+	-	-	+	+	-
11.	Starch	-	-	-	-	-	-	-	-
12.	Terpenoids	++	++	+++	-	+	+	-	+
13.	Resins	++	++	+++	+	+	+	+	+

Chemical Analysis :(TLC analysis, Heavy metals, Aflatoxins, Fluorescence behavior)

Chloroform, Ethyl acetate, Ethanol and Acetone extract of the drug were carried out TLC analysis whose chromatogram was developed using the solvent toluene: ethyl acetate: Methanol (7:2:1) and detected under the UV 366nm and derivatizing with anisaldehyde sulphuric acid and observed in visible region and at 580nm clearly shown different spots with Rf values as shown in the figure 4. The study carried out on heavy metals such as cadmium, lead, mercury and arsenic were found to be absent as given in table-6. Similarly Aflatoxins were analyzed and found to be absent as given in the table-7 inferring the drug to be safe and non toxic. Powdered drug was screened for fluorescence characteristic with or without chemical treatment. The observations pertaining to their colour in daylight i.e., visible region and under ultra-violet light were noticed and are presented in the table-8. Fluorescence analysis of powdered drug extracts in different solvents was observed and reported in the table-9.

Table.3. Heavy Metal Analysis

S. No	Parameter analyzed	Results found	Permissible limits as per WHO
1	Arsenic	Nil	Not more than 3.0 ppm
2	Cadmium	Nil	Not more than 0.3 ppm
3	Lead	Nil	Not more than 10.0 ppm
4	Mercury	Nil	Not more than 1.0 ppm

Table.4. Aflatoxin Contamination

S.No	Parameter analyzed	Results found	Permissible limits as per WHO
1	B1	Nil	Not more than 0.50 ppm
2	B2	Nil	Not more than 0.10 ppm
3	G1	Nil	Not more than 0.50 ppm
4	G2	Nil	Not more than 0.10 ppm

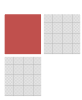


Table-5. Fluorescence analysis of powdered drug

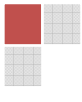
S.No	Reagents	UV light		Visible light
		Short 254nm	Long 366nm	
1.	Powder as such	Black	Brown	Brown
2.	Powder treated with 1N NaOH in Methanol	Black	Light green	Light brown
3.	Powder treated with 1N NaOH in Water	Black	Dark green	Dark brown
4.	Powder treated with 1N HCl	Black	Grey	Dark brown
5.	Powder treated with 50% HNO3 aqueous	Black	Black	Brown
6.	Powder treated with 50% H_2SO_4 aqueous	Black	Black	Black
7.	Powder treated with Glacial Acetic acid	Black	Black	Brown

Table-6. Fluorescence analysis of powdered drug extracts in different solvents:

S.No.	Extraction Solvent	UV light		Visible light
		Short 254nm	Long 366nm	
1.	Acetone Extract	Black	Blue	Light brown
2.	Alcoholic Extract	Black	Light blue	Light brown
3.	Chloroform Extract	Black	Light blue	Dark brown
4.	Petroleum ether extract	Black	Light green	Light brown
5.	Methanol	Black	Blue	Light brown
6.	Ethyl Acetate	Black	Blue	Light brown
7.	Distilled water	Black	Light green	Dark brown

Table 7. TLC profile of different extract of *Shorea robusta* Oleo resin along with R_f values and detection system.

S.no	Name of the extract	Solvent system	Detection system	No. of spots	R_f values
1.	chloroform extract	Toluene: Ethyl acetate: Methanol=7:2:1	Anisaldehyde sulphuric acid and scanned at 580nm	5	0.05 0.27 0.48 0.76 0.94
2.	Ethyl acetate extract	Toluene: Ethyl acetate: Methanol=7:2:1	Anisaldehyde sulphuric acid and scanned at 580nm	5	0.06 0.27 0.48 0.76 0.99
3.	Ethanol extract	Toluene: Ethyl acetate: Methanol=7:2:1	Anisaldehyde sulphuric acid and scanned at 580nm	5	0.05 0.27 0.49 0.75 0.99
4.	Acetone	Toluene: Ethyl acetate: Methanol=7:2:1	Anisaldehyde sulphuric acid and scanned at 580nm	5	0.06 0.26 0.45 0.75 0.99



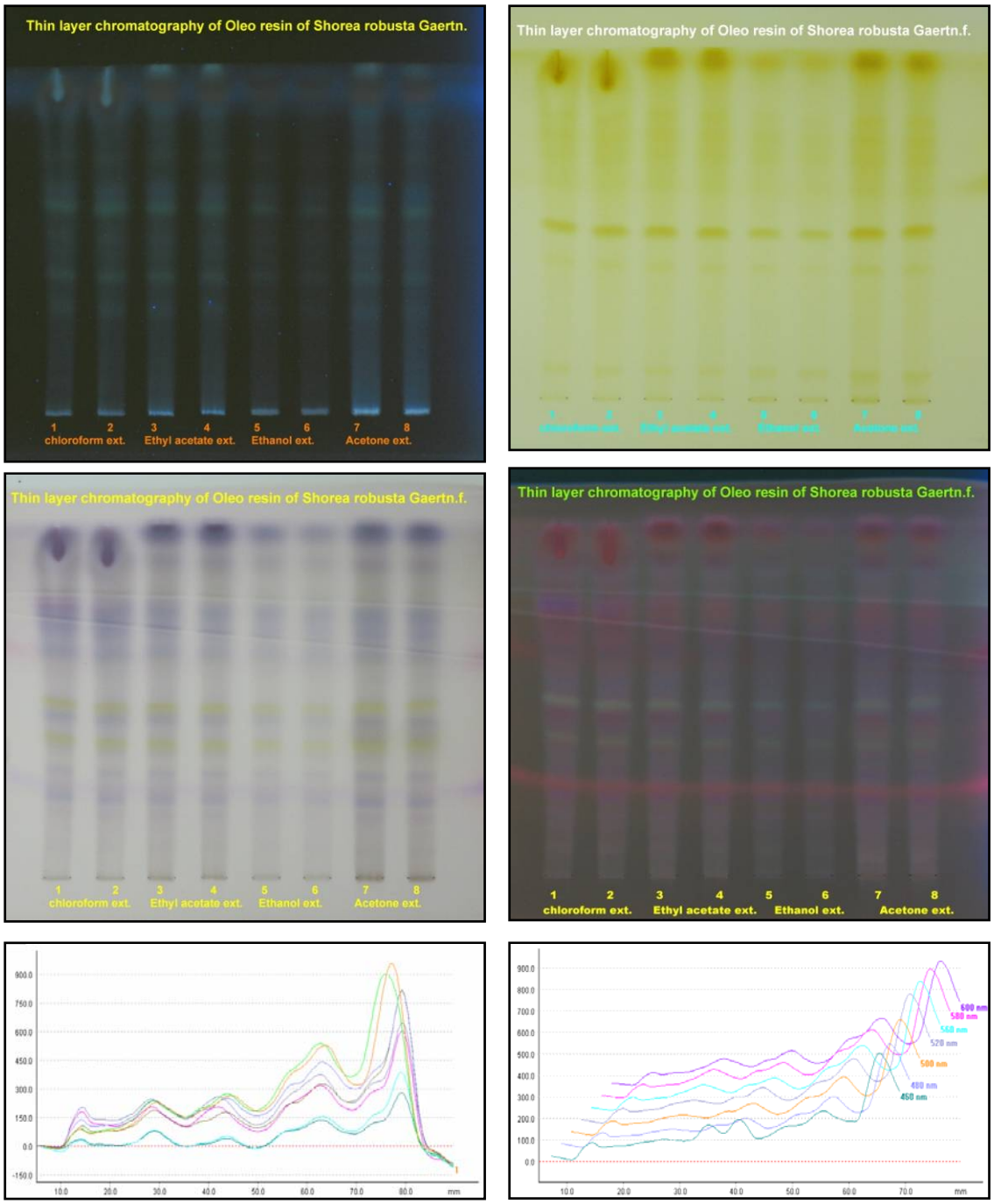


Figure. 6(a-d) Tlc plates photograph at UV 366nm, Under Iodine vapours, Derivatized with anisaldehyde sulphuric aid, and after derivatization & observed at UV 366nm. 6(e) Overlay densitogram of the shorea robusta oleo resin of chloroform, ethyl acetate, ethanol and acetone extracts. 6(f). Multiwavelength scan has carried out and found the wave length having better resolution and intense peak at 580nm in all the components of the sample.

Conclusion

Based on the results obtained under study for Oleo resin of *Shorea robusta* for Physico- chemical analysis, which is very much supportive in establishing the standards along with the other parameters such as macroscopic, microscopic, fluorescence behavior as reported in the present investigation including heavy metals and aflatoxins as found to be absent indicating the drug is safe. Phytochemical screening also reveals the presence flavonoids, saponins, steroids, tannins, phenols etc mainly triterpenoids which plays the prominent role for their therapeutic potential in the drug as reported in the literature. Consequently the drug was brought up in determining and ascertaining its quality standard and also developed the HPTLC fingerprint profile of the drug in the different solvent extracts which helps to identify and to quality control check**.** Thus, the study is likely to help in the quality assurance and standard reference for the drug in future use. The bioactive natural products may provide new drugs for various skin diseases.

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