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Pharmacognostical Evaluation on the leaves of *Wrightia tinctoria (Roxb) R.Br.*

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Abstract

***Plan**: A preliminary Pharmacognostical study on the leaves of Wrightia tinctoria (Roxb) R.Br.*
***Methodology**: The Wrightia tinctoria (Roxb) R.Br., leaves were collected dried and studied to determine various parameters of Pharmacognostical standards such as ash values, extractive values, phytochemical tests and microscopical characters of leaf powder. The shade dried powder and various solvent extracts (viz., methanol, 70% ethanol, aqueous, dichloromethane, chloroform, ethyl acetate and petroleum ether) have been analyzed for their phytoconstituentsand fluorescence characters. The methanolic extract was found to contain presence of triterpenes.*
***Outcome**: The data generated for the Pharmacognostical evaluation on Wrightia tinctoria leaves may be useful for establishing the standardization protocols. The HPTLC analysis data indicated that the collected Wrightia tinctoria leaves contain 47.6mg of lupeol/ g of the total methanolic extract.*
***Key words:** Wrightia tinctoria. Pharmacognostical evaluation, Phytochemical tests, HPTLC quantitation, lupeol*

1. Introduction

Wrightia tinctoria (Roxb) R.Br., (Family: Apocynaceae) is found in central India, Western Peninsula, Coromandal coast ,Coimbatore, Godavery district, Rajputana, Deccan, Konkan, Western ghats of Madras Presidency, Ceylon, Burma etc., which is commonly used in Indian system of medicine. It is known as Vetpalai in Tamil, Indigo –Plant in English and Indrajava, Hyamaraka in Sanskrit respectively.[1] It is a densely foliaceous deciduous tree which grows in plains and slopes of Shevaroy hills. Yellow (or) light brown glabrous (or) puberulous branchlets.

The leaves are 6-14 cm long and 3-6 cm broad, elliptic lanceolate (or) oblong-lanceolate, acuminate glabrous or the young leaves puberulous beneath, base acute or rounded ; 6-12 pairs of main nerves scaly bark of reddish-brown colour and smooth appearance as compared with *Holarrhena* bark which has white jasmine like flowers with a fragrant odour. Double follicle, apically connate and cylindrical. Seeds is with a tuft of deciduous coma at the tip.[2] It is well known for its medicinal effect and is being traditionally used for the treatment of various aliments such as anthelmintic, antidiarrhoeal, antidysentric, astringent, febrifuge, tonic, seeds are given in flatulence.

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Leaves are chewed to relieve toothache and the bark is used as antidysenteric[3]. Lakshmidevi *et al* (2010) reported [12] the LC_{50} value of the methanolic leaf extracts of *Wrightia tinctoria* as 471.604 µg /ml by Brine shrimp assay method. Mukerji B (1953) reported[13] the presence of saponins in the leaves of *W.tinctoria*. The total amount of triterpene saponins in the leaves *W.tinctoria* was reported[14] by Mahendra S. K *et al* (2009). In the light of the above facts, we have decided, to evaluate the *W.tinctoria* leaves systematically and to quantify the amount of the major active triterpenoid compound, lupeol in it.

2. Materials and methods

The crude drug (fresh leaves) under the name vetpalai were collected from Shevaroy hills and it was identified and authenticated (BSI/SC/5/23/08-09/TECH-741) by the Botanical Survey of India, Tamil Nadu Agricultural University, Coimbatore.

2.1. *Macroscopical characters.*

The macroscopical characters such as colour, odour, taste, shape, margine, apex, base, surface and size of *Wrightia tinctoria* leaf were observed.

Table 1: Macroscopical characters of *Wrightia tinctoria* leaves

S.No.	*Macroscopic Parameters*	*Observation*
1.	Colour	DarkGreen
2.	Taste	Bitter
3.	Odour	Characteristic
4.	Shape	Elliptic – ovate (or) Lanceolate (or) Oblong-lanceolate
5.	Margin	Entire
6.	Apex	Acuminate
7.	Base	Acute (or) Rounded
8.	Surface	Glabrous (or) the young leaves puberulous beneath
9.	Size	7.5 -15 (L)by 2.5-5.6 (B) cm

2.2. *Ash and Extractive values*[4]

2.2.1. Determination of Total Ash

The total ash was determined by incinerating 20 gm of air-dried coarsely powdered drug in a tared silica crucible which was previously ignited and cooled before weighing. The ignition was repeated until constant weight was obtained. The percentage of ash with reference to air-dried drug was calculated.

2.2.2. Acid-Insoluble Ash

The ash was boiled with 25 ml 2M HCl for 5 minutes in a boiling tube and filtered through a Whatman No. 42 filter paper. The residue was washed with hot water, ignited to ash, cooled in a dessicator and weighed .The percentage of acid- insoluble ash with reference to air-dried drug was calculated.

2.2.3. Water-soluble Ash

The ash was boiled for 5 minutes with 25 ml of distilled water and filtered through a Whatman No. 42 filter paper. The residue was washed with hot water, ignited to ash, cooled and weighed. This weight was subtracted from the weight of ash and the difference in weight representing the water soluble ash. The percentage of water soluble ash with reference to air-dried drug was calculated.[5,6]

2.3. *Extractive values*

About 5 gm of powdered drug was weighed and 100 ml of 90% alcohol was added and shaked frequently for 23 hrs and set asaid for 1 hr. 25ml of the filtrate was evaporated to dryness on a water-bath and complete the drying in an oven at 100^{o} C. Cool in a dessicator and weighed. Thus the percentage w/w alcohol soluble extractive values were calculated with reference to air - dried crude drug powder. Chloroform water and ether was used instead of alcohol for water soluble and ether soluble extractive values.[7]

Table 2: Ash and Extractive values of *Wrightia tinctoria* leaves

S.No.	*Parameters*	*Percentage w/w*
	Ash values	
1.	Total ash	15.23
2.	Water Soluble ash	10.28
3.	Acid insoluble ash	4.28
	Extractive values	
5.	Alcohol soluble	12.5
6.	Water soluble	10.7
7.	Ether soluble	7.2

Table 3: Preliminary Phytochemical screening of various extracts of Wrightia *tinctoria* leaves

Phyto constituent	70% Ethanol	Methanol	Aqueous	EthylAcetate	Chloro-form	Petroleum Ether	Dichloromethane
Carbohydrates	+++	+++	+++	-	-	-	-
Tannins	+++	++	++	+	+	-	-
Alkaloids	++	+++	-	+	++	-	+++
Flavonoids	++	++	-	++	+	+	-
Triterpenoids	++	+++	+	++	++	-	-
Saponins	+++	++	+++	-	-	-	-
Steroids	++	+++	-	++	-	-	-
Amino acids	-	+	-	-	-	-	-
Unsaturated Hydrocarbons	++	++	+	-	-	-	-
Glycosides	-	-	-	-	-	-	-

+++: High ++: Intermediate +: Low - : Negative

2.4. *Examination of the Drug powder*

Shade dried leaves were ground with wood - grinder and sifted through 40 mesh sieve. The ingredients of powder were observed under microscope.

The powder was treated with different chemical reagents and observations were made.[9] Table-4

Table 4: Data showing the behavior of *Wrightia tinctoria leaf* powder with different chemical reagents.

S.No	*Sample Treatment with*	*Observation*
1.	Powder as such	Light Brownish- Green
2.	IN HCl	Pale Brown
3.	IN NaOH	Light Yellowish Green
4.	5% KOH	Yellow
5.	5% $Fecl_3$	Reddish Brown
6.	5% Iodine	Cherry Red
7.	Picric acid	Pale Yellowish Brown
8.	HNO_3 + Ammonia solution	Orange Red
9.	IN HNO_3	Pale red
10.	Conc. H_2SO_4	Reddish Brown
11.	Conc. HCl	Bright Green
12.	Conc. HNO_3	Red
13.	Glacial acetic acid	Greenish Brown
14.	Ammonia solution.	Red

2.5. Fluorescence analysis

Small quantity of drug powder mounted in different solvents was analyzed under UV and visible light[10, 11] also Fluorescence Nature of solvent extracts has been analyzed. Table-5

Table 5: Fluorescence analysis of *Wrightia tinctoria* leaf powder under ultra violet (UV) radiation

S.No	*Treatment*	*Observation*		
		Short (254nm)	*Long (366 nm)*	*Visible light*
1.	Powder as such	Green	Light Brownish Green	Light Brownish Green
2.	Powder + 1N Hcl	Pale Brown	Bright Green Fluorescence	Pale Brown
3.	Powder + 1N NaOH	Green Fluorescence	Yellowish green	Light Yellowish Green
4.	Powder + 50% HNO_3	Green Fluorescence	Light brown	Pale Brown
5.	Powder + 50% H_2SO_4	Bright Green Fluorescence	Light brown	Light Brown
6.	Powder + Methanol	Green Fluorescence	Brown	Pale Red
7.	Powder + Acetic acid	Brownish black	Dark Brown	Greenish Brown
8.	Powder + Picric acid	Bright Green Fluorescence	Pale Yellow Brown	Pale yellow Brown
9.	Powder + 1 N NaoH in methanol	Bright Green Fluorescence	Reddish Brown	Reddish Brown
10.	Powder + 5% $FeCl_3$	Bottle Green	Brown	Reddish Brown

Table 6: Fluorescence nature of difference solvent extracts of *Wrightia tinctoria* leaves under ultra violet (UV) light

No	*Extract*	*Observation Wrightia arborea*		
		Short (254nm)	*Long (366 nm)*	*Visible light*
1.	Water	Yellowish green	Purple	Dark Brown
2.	70% ethanol	Greenish Brown	Dark Blue	Brownish Black
3.	Ethanol	Dark Reddish Brown	Dark Brown	Brown
4.	Methanol	Green	Dark Brown	Bright Green
5.	Petroleum ether	Greenish yellow	Reddish Brown	Light Browm
6.	Chloroform	Dark Green	Dark Brown	Dark Reddish brown
7.	Ethyl acetate	Green	Dark Reddish Brown	Dark Brown
8.	Dichloromethane	Greenish Brown	Brown	Reddish Brown

2.6. *Tissues of diagnostic importance*

The course free flowing crude drug powder was light brownish green colour, with bitter taste characteristic odour. Small amount of the powdered material sieved through 40 meshes was placed on a microscopic slide; mixed with few drops of 40% w/v aqueous chloral hydrate solution heated gently. Few drops of 1% alcoholic phloroglucinol was added to this and warmed by mixing with or drop of concentrated hydrochloric acid, mounted with glycerin water, observed under microscope showed the presence of paracytic stomata at lower epidermis.

Narrow thick walled laticifers dispersed in the ground tissue and circular less compact parenchyma. Several 3 or 4 thick walled angular xylem elements was showed bowel shaped vascular strand. Phloem occurs in circular discrete masses both along the inner and outer side of xylem .

The ground tissue consists of homogeneous, circular, thin walled and less compact parenchyma cells dispersed in it. Collenchyma cells of 6-8 layers present in the adaxial bundle of epidermis. Epidermis was of apostomatic type at adaxial side and had small fairly thick walled amoeboid outline; with anticlinical and wavy walls.

Fairly, abundant crystals in the mesophyll of leaf, randomly distributed were of in the form of Druses or sphaero type. Very rarely exhibited epidermal trichomes are of non - glandular type. They were multicellular, uniseriate and unbranched with vertically (2-4 cells) elongated rectangular and thick walled cells with length upto 120 µm long and 20 µm thick with a blunt tip.

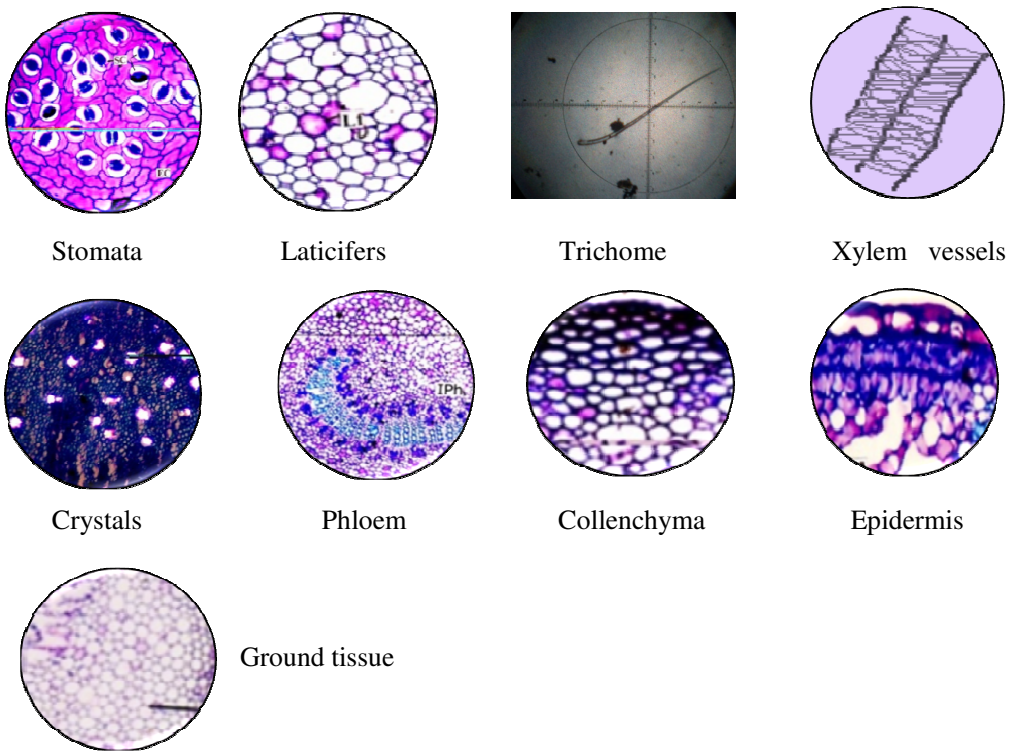
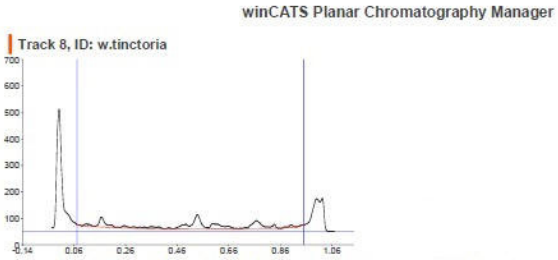


Figure -1: Powder Characters of *Wrightia* tinctoria Leaf

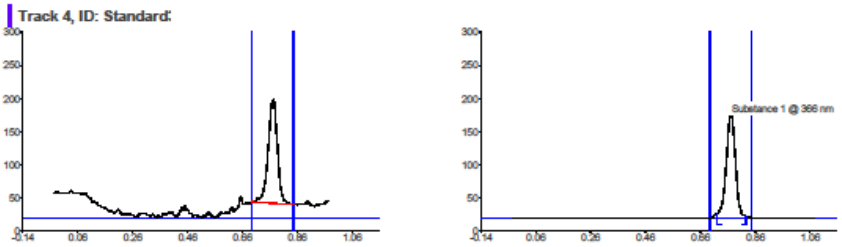
2.7. *HPTLC fingerprinting & quantitation studies*

HPTLC system equipped with Linomat v applicators, TLC scanner, controlled by wincats-4 software were used for the HPTLC evaluation of *W.tinctorea* leaf methanolic extract (WTLM) and quantitation of lupeol in the total extract. All the solvents used, were of HPTLC grade obtained from Merk. Lupeol was used as the marker compound. A total of six spots were observed in the chromatogram and the spot (S_5) having R_f value (0.77) is found matching with the marker lupeol (0.77), Fig.2 and 3. The densitometric evaluation of the active compounds present in WTLM and lupeol was performed at 366 nm using tungsten lamp with camag scanner II in conjunction with CATS software for quantitation. Chromatography was performed on silica gel F_{254} HPTLC plates (Merk 5554). The mobile phase was toluene: ethyl acetate: methanol (15:3:1.5). The plates were developed to a distance of 8.2cm in a Camag twin trough chamber, previously saturated with the mobile phase. After development and drying the plates were analyzed by HPTLC scanner device. The results are tabulated in table VII. The plates were sprayed with natural product: polyethylene glycol reagent and photographed under UV light. The data is tabulated in table VII and VIII.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.14	0.6	0.17	37.2	21.45	0.19	7.3	641.3	17.38	Substance 1
2	0.47	7.8	0.49	17.7	10.23	0.50	10.5	375.8	10.18	Substance 5
3	0.51	10.6	0.54	54.0	31.18	0.57	5.5	1152.0	31.21	Substance 2
4	0.58	5.9	0.60	19.6	11.31	0.63	8.4	526.4	14.26	Substance 6
5	0.73	2.1	0.77	29.4	16.99	0.80	5.4	819.5	22.21	Substance 4
6	0.82	4.4	0.84	15.3	8.84	0.85	0.0	175.6	4.76	Substance 11

Fig.2and Table VII: HPTLC studies and quantitation of *W.tinctoria* leaf extract (WTLM) with chemical marker lupeol



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.72	6.0	0.77	156.6	100.00	0.82	1.8	4259.7	100.00	

Fig.3and Table.VIII, HPTLC of lupeol (marker compound)

3. Results

Consistent quality for products of herbal origin can only be achieved if the starting plant materials are defined in a rigorous and detailed manner. The Pharmacognostical evaluation on *Wrightia tinctoria (Roxb)* R.Br. comprising determination of parameters like macroscopical characters were observed as shown in (Table-1) Analytical parameters like ash and extractive values were carried out and the results were tabulated as shown in (Table-2). The phytoconstituents like carbohydrates, tannins, alkaloids, flavonoids, triterpenoids, saponins, steroids, amino acids, unsaturated hydrocarbons and glycosides in each extract were identified and the results were tabulated as shown in (Table-3). The behavior of powdered leaf drug with different chemical reagents had been observed as shown in (Table -4).

The fluorescence nature of leaf powder with different reagents under visible light and UV radiation were observed as shown in (Table -5) and the same for different solvent extracts like aqueous,70% ethanol, methanol, petroleum ether, chloroform, ethyl acetate and dichloromethane was observed as shown in (Table-6) .

Powdered drug showed paracytic type of stomata, druses or sphaero type of crystals, dispersed narrow thick walled laticifers, circular discrete masses of phloem, thick walled angular xylem, apostomatic type of adaxial epidermis, multicellular uniseriate trichomes and homogeneous less compact parenchyma cells (Fig-1). The amount of the marker compound lupeol was found to be 47.6 mg per gram of the total extract (WTLM).

4. Discussion

Characterization of an herbal drug is therefore essential to allow specifications to be established which are both comprehensive and relevant. The observations in the present study have brought out several diagnostic features of the leaf on the basis of which identification of the crude drug can be ascertained. As the drug has been standardized on the basis of certain Pharmacognostical characters, such as the powdered drug, besides the leaf characters, inference of other studies can serve characteristic features of the drug. Thus, the present study on Pharmacognostical characters of *Wrightia tinctoria* may be useful as supplement information with regard to its identification and shall be helpful in establishing the standardization criteria.

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