

Pharmacognostical and phytochemical evaluation of *Celastrus paniculata*

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ABSTRACT

The present study aimed at detailed pharmacognostic evaluation of the crude drug, *Celastrus paniculata*. Morphoanatomy of the leaves of *C. paniculata* was studied with the aim to aid pharmacognostic and taxonomic species identification using light and confocal microscopy, WHO suggested physico-chemical determinations and authentic phytochemical measures. Different extracts were prepared using the soxhlation method and these were used for phytochemical analysis and to determine various pharmacognostic parameters. The various measures were performed following the WHO guidelines. Various pharmacognostic parameters were reported following the WHO guidelines. The physico-chemical and morphological parameters presented in this paper may be proposed as parameters to establish the authenticity of *C. paniculata* and can possibly help to differentiate the drug from its other species.

Keywords: *Celastrus paniculata*, Celastrus, pharmacognosy, phytochemistry, standardization.

INTRODUCTION

Celastrus paniculatus Willd. (Celastraceae) is a large woody, climbing shrub, height up to 18 m with stem diameter up to 23 cm. It is distributed in a sub-Himalayan tract and almost all over India up to an altitude of 1800 m^{1,2}. A large climbing shrub with woody stem and elongate branches. *C. paniculatus* is a deciduous vine with stems up to 10 centimeters in diameter and 6 meters long with rough, pale brown exfoliating bark covered densely with small, elongated lenticles. The leaves are simple, broad, and oval, obovate or elliptic in shape, with toothed margins.^{3,4}

Celastrus paniculatus Willd. (Celastraceae) is widely known as "Malkangni". It is a large woody climber indigenous to India. The oil, obtained from the seeds of this plant, is largely used in Ayurvedic medicine for the treatment of rheumatism, as sedative and to strengthen the intellectual power and mental alertness.⁵

A triterpene compound lupeol isolated from pet. Ether extract of leaves of *Celastrus paniculata* was screened for wound healing activity¹³.

Identification of a new sesquiterpene polyol ester has been characterized as a new sesquiterpene polyol ester characterized as 1,6,8-triacetoxy-9-benzoyloxydihydro-agarofuran (1), along with the 3 known compds.: 1,6,8-triacetoxy-9-benzoyloxydihydro-agarofuran (2), angulatueoid C (3), and 1,6,8,14-tetraacetoxy-9-benzoyloxydihydro-agarofuran (4), was isolated from the CCl₄-sol. fraction of *Celastrus paniculatus* methanolic seed ext¹².

MATERIALS AND METHODS

Collection of plant material and its identification – The leaves of *Celastrus paniculata* were collected from M/S Sheikh International, Dindigul, Tamil Nadu, India, during

the month of August, 2010. The botanical identity of the plant was confirmed by Dr. H.B.Singh, National Institute of Science Communication and Information Resources, Delhi, India. A voucher specimen no. NISCAIR/RHMD/Consult/-2010-2011/1588/186 has been deposited at the Museum of the faculty of Pharmaceutical Science, Jodhpur National University, Jodhpur, Rajasthan, India.

Chemicals and instruments – Compound microscope, glass slides, cover slips, watch glass and other common glass ware were the basic apparatus and instruments used for the study. Microphotographs were taken using a Leica DMLS microscope attached with Leitz MPS 32 camera. Solvents viz. petroleum ether, benzene, chloroform, acetone, ethanol (99.9%), n-butanol and reagents viz. phloroglucinol, glycerine, HCl, chloral hydrate and sodium hydroxide were procured from Ranbaxy Fine Chemicals Ltd., Mumbai, India.

Macroscopic and Microscopic analysis – The macroscopy and microscopy of the leaf was studied according to the standard method⁸. For the microscopical studies, cross sections were prepared and stained as per the standard procedure¹¹. The micro-powder analysis was done according to the standard methods^{9,15}.

Physico-chemical analysis – The percentage of ash values and extractive values were performed according to the official methods prescribed⁶ and the WHO guidelines on the quality control methods for medicinal plant materials⁷.

Preliminary Organoleptic Examination

Size: A graduated ruler in millimeters was used for the measurement of the length and width of crude materials.

Colour: Untreated sample was examined under diffused daylight.

Preliminary organoleptic examination: (Table 1)

Table 1. Preliminary organoleptic examination

S. No.	Property	Characteristics of <i>C. bonduc</i> leaves
1.	Taste	Bitter , Pungent
2.	Colour	Pale green
3.	Odour	characteristic
4.	Size	Lamina length 10-12 cm, Breath 5.5- 5.9 cm.
5.	Surface characteristics	Smooth, glabrous above, glaucous beneath and Pubescent

Determination of extractable matter: (Table 2)

Table 2. Determination of extractable matter

Method	Extract	Weight of empty petridish (g)	Weight of petridish + dried extract (g)	Difference (g)	Extractable matter (mg/g)	Colour of extract
Hot	Pet. Ether	28.423	28.447	0.024	6	Grass green
	DCM	40.056	40.092	0.036	9	Dark green
	Ethyl acetate	54.383	54.423	0.04	10	Dull green
	Methanol	49.352	49.576	0.224	56	Bottle green
	Ethanol	43.543	43.713	0.17	42.5	Bottle green
	Aqueous	42.342	42.644	0.302	75.5	Dark green
Cold	Pet. Ether	45.543	45.554	0.011	2.75	Grass green
	DCM	52.251	52.284	0.033	8.25	Dark green
	Ethyl acetate	50.567	50.623	0.056	14	Dull green
	Methanol	43.262	43.452	0.19	47.5	Bottle green
	Ethanol	43.263	43.443	0.18	45	Bottle green
	Aqueous	50.611	50.854	0.243	60.75	Dark green

Weight of drug taken every time = 4 g

Table 3. Foaming index

Test tube	1	2	3	4	5	6	7	8	9	10
Ratio of water: extract	9:1	8:2	7:3	6:4	5:5	4:6	3:7	2:8	1:9	0:10
Height of foam (cm)	0	0	0.1	0.1	0.1	0.1	0	0	0	0

Height of froth was measured and it was noticed that no froth measured was more than 1cm therefore the foaming index is less than 100.

Swelling index: (Table 4)

Table 4. Swelling index

Time	Volume of plant material (ml)				Mean (ml)	Difference for 4g plant material D=(b-a) (ml)	Swelling Index for 1g (D/4) (ml)
Initially	40	40	40	40	40		
After 3hours	45	46	45	45.33	5.33	1.33	

Surface characteristics, texture: The material was touched to determine if it is soft or hard; bended and ruptured to obtain information on brittleness.

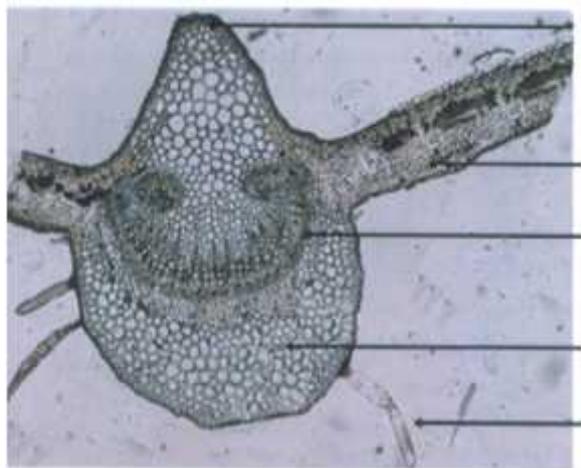
Odour: The material was powdered and the strength of the odour was determined whether none, weak, distinct or strong and then the sensation of odour whether aromatic, fruity, musty, mouldy, rancid, etc was observed.

Taste: Small amount of plant material was tasted and the taste was observed⁷.

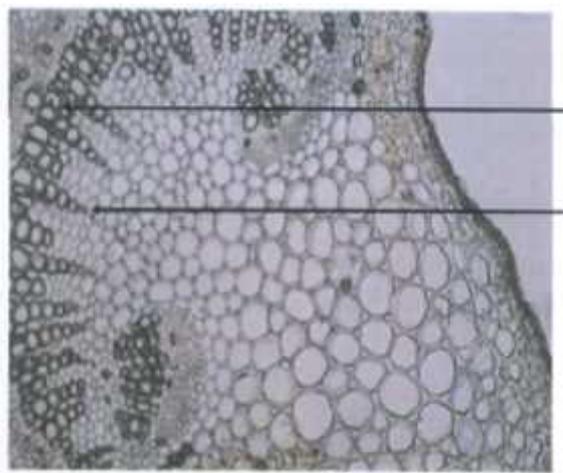
Determination of foreign matter: The plant samples were examined for the foreign matter⁷.

Determination of ash: The ash remaining after ignition of medicinal plant materials was determined by two different methods which measure total ash and acid insoluble ash.

Total ash: The ground air-dried material (2g) was accurately weighed and placed in a previously ignited and tared crucible. The material was spread in an even layer and was ignited by gradually increasing the heat to 500-600°C until it was white, indicating the absence of carbon. It was cooled in a desiccator for 30 minutes and weighed without delay. The content of total ash was calculated in mg per g of the air dried material⁷.

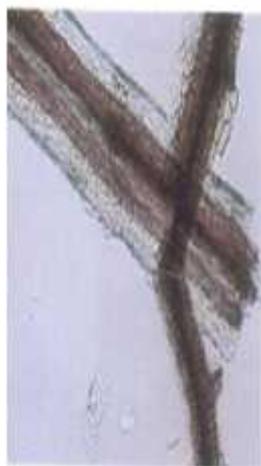


A



B

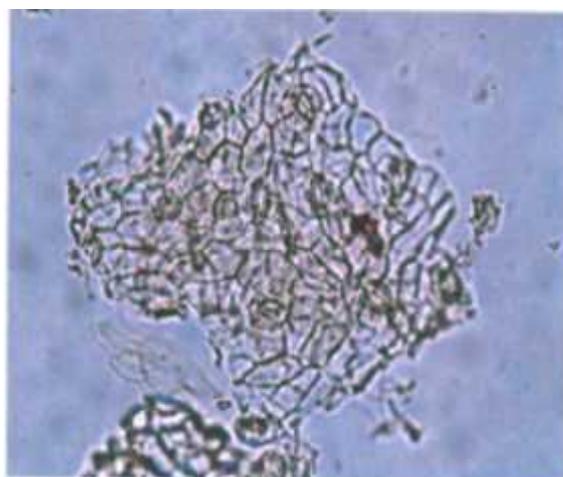
Plates A and B representative photomicrographs of T.S. of *Celastrus paniculatus* leaf



C



D



E

Plates C, D and E representative photomicrographs of powdered *Celastrus paniculatus*

Loss on drying: Air dried material was accurately weighed (4g) in a previously dried and tared weighing bottle. The sample was dried in an oven at 105^o C. It was dried until two consecutive weighings did not differ by 5 mg. The loss of weight was calculated in mg per g of air dried material⁷.

Determination of moisture content

Karl Fischer titration: Methanol (20 ml) was added to the titration beaker. Main plug was connected to 230 volts AC. and mains switch was switched on. The burette stopcock on the main unit was turned on. The stirrer was turned on and the speed of stirring capsule with magnetic stirrer regulator was adjusted. Red neon light indicated the instrument to be switched on. The timer knob was set to 20 seconds on the dial. The stopcock was kept closed and then the button to start the titration was pressed momentarily. The yellow neon light went off immediately and there was a click sound of solenoid valve indicating that the solenoid valve was energized. Then the stopcock of the automatic Karl Fischer burette was opened slowly so that Karl Fischer reagent from the bent tube was added drop wise to the methanol in the beaker. With addition of

every Karl Fischer reagent drop, the pointer swung to right side and came back again to its zero position. The solenoid valve kept on switching off and on until complete moisture in the methanol was removed. At the end point, the meter pointer remained on the right side. The reading on the Karl Fischer burette was noted and Karl Fischer factor was calculated as

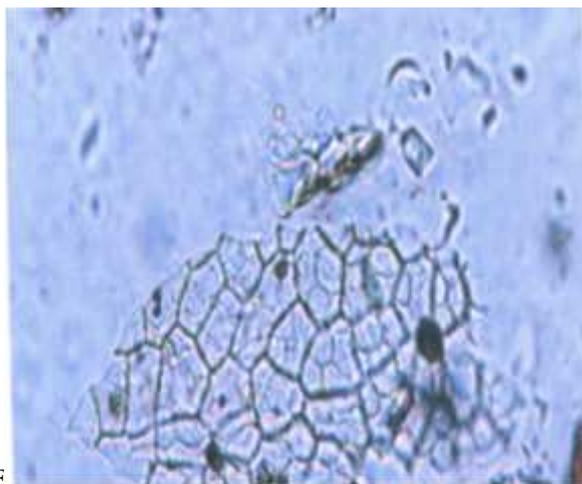
$$\text{KarlFischerfactor} = \frac{15.66}{\text{Volume in ml of KarlFischerreagent used}}$$

A specified quantity (50 mg) was weighed and was added to the methanol in the beaker. The button to start titration was pressed and the sample was titrated. The moisture content was calculated by the formula

$$\text{Moisture content} = \frac{100 \times \text{Karl Fischer reagent reading} \times \text{Factor}}{\text{Weight of sample in mg}}^{18}$$

Determination of extractable matter

Hot extraction: Coarsely powdered air-dried material (4.0g) was accurately weighed and placed in glass-stoppered conical flask. 100ml of water was added and total weight including the flask was noted. It was shaken well and allowed to stand for 1 hour. Reflux condenser was attached to the flask and gently boiled for 1 hour



F
Plate F representative Photomicrograph of leaf Epidermis along with underlying palisade cell



G
Plate G representative photomicrograph of surface view of leaf lamina showing vein islets and vein termination no.

H

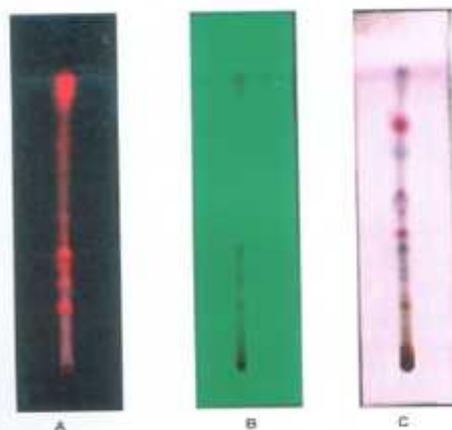


Plate H representative photomicrograph of thin layer Chromatography of methanol extract of *Celastrus paniculatus* leaf

cooled and weighed. Original total weight was readjusted with the solvent specified in the test procedure for the plant material concerned. It was then shaken well and filtered rapidly through a dry filter. Filtrate (25ml) was transferred to a tared flat-bottomed dish and evaporated to dryness on a water bath. Then it was dried at 105°C for 6 hours and then cooled in a desiccator for 30 minutes and then weighed without delay. The content of extractable matter in mg per g of air dried material was calculated.

Cold extraction: Coarsely powdered air-dried material (4.0g) was accurately weighed and placed in glass-stoppered conical flask. It was macerated with 100ml of the solvent specified for the plant material for 6 hours, shaken frequently and then allowed to stand for 18 hours. It was filtered rapidly taking care not to lose any solvent. Filtrate (25ml) was transferred to tared flat-bottomed dish and evaporated to dryness on a water bath. It was dried at 105°C for 6 hours, cooled in a desiccator for 30 minutes and weighed without delay. The content of extractable matter in mg per g of air dried material was calculated.

Foaming index

Plant material (1g) was reduced to a coarse powder, weighed accurately and transferred to a 500ml conical

flask containing 100ml of boiling water. It was maintained at moderate temperature for 30 minutes. It was cooled and filtered into a 100ml volumetric flask and added sufficient water through the filter to dilute it. The decoction was poured into 10 stoppered test-tubes in successive portions of 1ml, 2ml, 3ml, 4ml, 5ml, 6ml, 7ml, 8ml, 9ml and 10ml. The tubes were stoppered and shaken in lengthwise motion for 15 seconds, two shakes per second and allowed to stand for 15 minutes and the height of foam was measured. The results were assessed as follows:

- i. If the height of the foam in every tube was less than 1cm, the foaming index would be 100.
- ii. If the height of foam of 1cm was measured in any of the test tubes, the volume of plant material decoction in that tube would be used to determine the index. If that tube was the first or second tube in a series, an intermediate dilution would be prepared in the same manner to obtain a more precise result.
- iii. If the height of foam was more than 1cm in every tube, then the foaming index would be over 1000. In this case, determination would be repeated using a new series of dilutions of the decoction in order to

Qualitative chemical examination of extracts:

Phytochemical screening (Table 5)

Table 5. Phytochemical screening

S. No.	Chemical constituents	Tests	Extracts			
			Pet. Ether	DCM	Ethyl acetate	Methanol
1.	Alkaloids	Mayer's test	-	-	-	+
		Dragendorff's reagent test	-	-	-	-
		Wagner's test	-	-	-	-
		Hager's test	-	-	-	+
2.	Carbohydrates	Molisch's test	-	-	-	-
		Benedict's test	-	-	-	-
		Fehling's test	-	-	-	-
3.	Glycosides	Modified Borntrager's test	-	-	-	-
		Legal test	-	-	-	-
4.	Phytosterols	Salkowski test	-	-	+	++
		Tshugajeu test	-	-	-	+
5.	Phenols	Ferric Chloride test	-	-	-	+
6.	Saponins	Foam test	-	-	-	-
7.	Tannins	Gelatin test	-	-	++	+
8.	Diterpenes	Copper Acetate test	-	-	-	-
9.	Resins	Acetone water test	-	-	-	+
10.	Flavonoids	Alkaline reagent test	-	-	-	-
		Zinc Hydrochloride reduction test	-	-	-	++
		Lead Acetate test	-	-	-	-
		Shinoda test	-	-	-	++
		Xanthoproteic test	-	-	-	-
11.	Amino acids and proteins	Ninhydrin test	-	-	+	+
		Biuret test	-	-	-	-

iv. obtain a result.

Foaming index was calculated using the following formula

$$\frac{100C}{a}$$

Where a= the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1 cm was observed.

Determination of swelling index: Powdered plant material (4g) was accurately weighed and put into a 100ml glass-stoppered measuring cylinder. Water (100ml) was added into it and shaken thoroughly after every 10 minutes for an hour. Then the suspension was allowed to stand for 3 hours at room temperature. The volume occupied by the swelled plant material was measured in ml. This was repeated for three times and mean value was calculated for 1g of plant material.

Preliminary phytochemical evaluation: Preliminary phytochemical screening was carried out by using standard procedures without spray reagent^{10,16}.

Successive solvent extraction was performed on small scale with coarsely powdered dried plant material (22g). The plant material was first soxhleted with petroleum ether (300ml) at 60°C for 3hrs and then the plant material was dried at room temperature. The dried plant material was soxhleted with DCM (300ml) at 40°C for 3hrs and then it was dried at room temperature. Again the same process was repeated for ethyl acetate at 77°C and

methanol at 70°C. Different extracts of plant material obtained were subjected to various chemical tests to detect the chemical constituents present in them.

Chromatographic studies

TLC plates- Silica gel GF 254 grade (E merck) was used to prepare TLC plates (8.5 X 20).

Solvent system- Toluene: chloroform: methanol (4:4.7:1.3)

Visualization - Plates were observed under UV at 254nm.

The sample was spotted on the plate and was allowed to dry it for few minutes. Then the solvent system was prepared and allowed to stabilize for 10 min. Then the plate was dipped in the solvent chamber and allowed it to run up to three fourth of the plate. Then it was removed and was air dried. The plate was kept in the UV chamber to visualize the spots. The Rf was calculated²⁰.

Microscopic studies of *C.paniculatus*

Study of transverse section: Leaves of *C. paniculatus* were boiled with water until soft. Free hand sections were cut, transferred on slides, cleared by warming with chloral hydrate and mounted in glycerine. Plate 1 shows the representative photomicrograph of transverse section of leaf of *C. paniculatus*^{8,21-26}.

Powder microscopy: Powder *C.Paniculatus* leaves were cleared with chloral hydrate, mounted in glycerin and observed under the microscope. The powdered plant material stained separately with phloroglucinol, HCl and

Thin Layer Chromatography: (Table 6)
Table 6. Thin Layer Chromatography.

S. No.	Extract	Distance travelled by solute	Solvent front	R _f value	Colour of spots	
					Visible	Ultra violet
1.	Petroleum ether	12.7	12.9	0.98	Dark green	Fluorescent pink
2.	DCM	9.3	12.9	0.72	Translucent	Light brown
		12	12.9	0.93	Dark green	Fluorescent pink
3.	Ethyl acetate	12.7	12.9	0.98	Dark green	Fluorescent pink
		8.5	12.9	0.66	Translucent	-
		9.6	12.9	0.74	Translucent	-
		12.6	12.9	0.98	Light green	Fluorescent pink
		12.8	12.9	0.99	Dark green	-
4.	Methanol	1.1	12.9	0.08	-	Fluorescent yellow
		1.8	12.9	0.14	-	Fluorescent yellow
		5.2	12.9	0.40	Translucent	Brown
		6.2	12.9	0.48	Translucent	Light brown
		7.5	12.9	0.58	Translucent	Light brown
		9.4	12.9	0.73	Translucent	Light brown
		10.4	12.9	0.80	Translucent	Light brown
		12.7	12.9	0.98	Dark green	Fluorescent pink

iodine solution was also examined. Plates shows representative photomicrographs of various microscope features^{8,21-26}.

RESULTS

Determination of foreign matter: Foreign matter like soil particles, hair, other plant materials, insects and feathers were separated manually.

Determination of total ash: Total ash = 60.5 mg/g

Determination of acid insoluble ash: Acid insoluble ash = 38.3 mg/g

Determination of water and volatile matter: Loss on drying = 86.75 mg/g

Determination of moisture content: Karl Fischer Titration

KF factor = 15.66 / KF ml on the burette

KF factor = 15.66 / 13 = 1.2046

Moisture content = 100 x KF reagent reading (ml) x Factor / Weight of sample in mg

Moisture content = 100 x 1.5 x 1.2046 / 50 = 3.614 %

DISCUSSION

The physicochemical, microscopical and morphological parameters presented in this paper may be proposed as parameters to establish the authenticity of *C. paniculata* and can possibly help to differentiate the drug from its other species. This study will play vital role in future studies for the isolation of its various constituents which may prove to be pharmacologically active.

CONCLUSION

The detailed study of *C. paniculata* leaves provides the pharmacognostic data in the form of various values which is helpful in its standardization.

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