Pharmaceutical Standardization

Phytochemical and analytical evaluation of *Jyotishmati* (*Celastrus paniculatus* Willd.) leaf extracts

Monojit Debnath, Moulisha Biswas, Vinay J. Shukla[1], K. Nishteswar[2]

Bengal Institute of Pharmaceutical Sciences, Nadia, West Bengal, [1]Pharmaceutical Chemistry Laboratory, [2]Department of Dravyaguna, Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India

Abstract

Background: *Jyotishmati* (*Celastrus paniculatus* Willd.) is a woody climber belongs to the family Celastraceae; a well known herbal nootropic, distributed through the tropical and subtropical regions of India. Its leaves are used in eye disease and headache. Very low qualitative and quantitative information about leaves have been documented to establish its quality and purity. **Aim:** Present study was conducted to evaluate physicochemical, phyto-chemical and HPTLC analysis of different solvent extracts of the *C. paniculatus* leaves. **Results:** Physico-chemical analysis revealed loss on drying 13.05% w/w, total ash value 16.08% w/w, acid insoluble ash 0.386% w/w, water-soluble extractive 14.22% w/w, alcohol-soluble extractive 9.91% w/w, chloroform-soluble extractive 7.75% w/w and ether-soluble extractive 4.74% w/w. Phytochemical screening showed the presence of steroid and terpenoid in the both pet. ether and ethyl acetate extracts while methanol extract possessed steroid, terpenoid, carbohydrate, alkaloid, saponin, and phenolic compounds. **Conclusion:** The observations made in this study may help to develop the standards of qualitative and quantitative parameters with regards to identification, quality and purity of *C. paniculatus* leaf.

Key words: *Celastrus paniculatus*, *Jyotishmati* leaf, high-performance thin layer chromatography, phytochemistry

Introduction

Jyotishmati (*Celastrus paniculatus* Willd.) is a woody scrambling or climbing polygamodioecious type of shrub, which with assistance from a nearby tree, climbs up to over 10 m. It occurs almost throughout India ascending to an altitude of 1800 m in the subtropical Himalayas. The seed oil and fruit is commonly used for its tranquillizing, sedative, wound-healing etc., activities.[1,2] The Ayurvedic application is mostly for its *Medhya* action (brain tonic).[1] *C. paniculatus* is one of such classical drugs which is being used by the tribal people from different regions of India, mainly Maharashtra, Orissa, and Andaman and Nicobar group of Islands. The bark is abortifacient, depurative, and used as a brain tonic. The leaves are emmenagogue and the leaf sap is a good antidote for opium poisoning. The seeds are acrid, bitter, thermogenic, emollient, stimulant, intellect-promoting, digestive, laxative, emetic, expectorant, appetizer, aphrodisiac, cardiotonic, anti-inflammatory,[3] diuretic, diaphoretic, febrifuge and tonic, and can treat abdominal disorders, leprosy, skin diseases, paralysis, asthma, leucoderma, cardiac debility,

inflammation, nephropathy, amenorrhea, dysmenorrhea. The leaves contain alkaloids, a glycoside and coloring matter, whereas the oil extracted from seeds contains sterols, alkaloids, and a bright coloring matter, Celapanin, Celapanigin, Celapagin, Celastrine and Paniculatine are some of the important alkaloids present in the seeds.[1] The oil also contains sesquiterpene like dipalmitoyl glycerol and alkaloids. Folklore experience is that the *Jyotishmati* leaf has good analgesic activity.[4]

With increasing demand for safer drugs, attention has been drawn to the quality, safety, efficacy and standards of the Ayurvedic drugs.[5] Hence, there is a need for standardization and development of reliable quality protocols for Ayurvedic drugs using modern techniques of analysis.[6] A detailed phytochemical and analytical investigation has not been performed till now on the leaves of *C. paniculatus*. Keeping this in view, present study was carried out to evaluate physicochemical, phyto-chemical and HPTLC analysis of different solvent extracts of the *C. paniculatus* leaves.

Materials and Methods

Plant material

The mature leaves of *C. paniculatus* (Celastraceae), were collected during November 2011 from Jamnagar, Gujarat, India. The plant material was taxonomically identified at the Central

Address for correspondence: Mr. Monojit Debnath, Assistant Professor, Bengal Institute of Pharmaceutical Sciences, Kalyani, Nadia - 741 235, West Bengal, India. E-mail: monodebnath@gmail.com

National Herbarium, Botanical Survey of India, Howrah, West Bengal, India. The voucher specimen (CNS/107/2011/Tech. II) was maintained in our research laboratory for future reference. The plant material was shade-dried with occasional shifting and then powdered with mechanical grinder, passing through sieve no. 40, and stored in an air-tight container.

Physico–chemical parameters

The powdered material was subjected to analysis of various physico-chemical parameters like loss on drying, ash value, total ash, acid-insoluble ash, water-soluble extractive, alcohol-soluble extractive, chloroform-soluble extractive, ether-soluble extractive, pH value, etc.[7]

Phytochemical screening

Phytochemical screening for carbohydrate, protein, amino acid, alkaloid, tannins, steroid, terpenoid, volatile oil, glycoside, fixed oil, etc., had been carried out.[8]

High-performance thin layer chromatography (HPTLC) analysis

HPTLC was carried out by the standard methods. The plate was developed in a solvent system of Toluene:Ethyl acetate (7:3).

Chromatographic conditions

A CAMAG (Switzerland) HPTLC system equipped with a sample applicator was used for the application of the samples. CAMAG TLC scanner 3, Reproster, and Winscat 4.02 were used for scanning of the plates. A CAMAG twin through a glass chamber was used for developing the plates.[9]

Chemicals

Precoated silica gel 60 F_{254} TLC aluminum plates (10 × 10 cm, 0.2 mm thick), AR grade toluene, ethyl acetate, methanol, petroleum ether were obtained from M/S Merck, Ltd., Mumbai.

Preparation of sample solution

The dried powdered material (23.2 g) was defatted with petroleum ether (60-80°C) by Soxhlet extractor and the percentage extractive value obtained was 4.74% w/w. The defatted powder material thus obtained was further extracted with ethyl acetate and methanol for 72 h in a percolator. The solvent was distilled off under reduced pressure and obtained semisolid mass was vacuum-dried using rotary flash evaporator to yield a solid residue and the percentage extractive values were 7.75% w/w and 9.91% w/w respectively. Sample solutions were prepared by dissolving the extracts in their respective solvents not following any quantitative manner but only by visual observation, because of the unavailability of the marker. Each test solution of 5 μl was spotted by CAMAG, Linomat 5 auto applicator. The plates were developed in a mobile phase of Toluene:Ethyl acetate (7:3) and scanned at 254 nm and 366 nm. The peak areas were noted and spectral comparison for the three types of extracts was studied.

Results and Discussion

Physicochemical data presented in Table 1 indicates that the loss on drying in the sample was 13.05% w/w, which shows that the value of moisture content is higher in the sample. The total ash value was 16.08% w/w, indicating presence of inorganic content in it. The contents, which are insoluble in acid, were 0.386% w/w.

The water-soluble extractive value is comparatively higher (14.22% w/w) than the alcohol-soluble extractive value (9.91% w/w), chloroform-soluble extractive value (7.75% w/w), and ether-soluble extractive value is 4.74% w/w. So, the solubility of the sample drug in water is higher than the other solvents. In the water extract, fat or resin content is very less, whereas alcohol-soluble matter like tannin, amino acid, alkaloids, etc., are high, and water-soluble matter like sugar, glycosides, carbohydrate, etc., are higher than the other. pH of 5% aqueous solution shows slightly acidic nature of leaf [Table 1].

The petroleum ether and chloroform extracts showed the presence of steroid, terpenoid, and fixed oil in very intense amount and methanol extract also showed the presence of steroid, terpenoid, fixed oil, carbohydrate, alkaloid, tannin, and glycoside in phytochemical analysis [Table 2].

In HPTLC analysis [Figures 1-3], the number of spots seen under 254 nm were 12, 12, and 17 for petroleum ether, ethyl acetate, and methanol extract respectively. The R_f values of major peak were 0.84 for petroleum ether extract; 0.54 for ethyl acetate extract; and 0.21 for methanol extract. When it was scanned at 366 nm 22, 20 and 20 spots were obtained for petroleum ether, ethyl acetate, and methanol extract respectively. The major peak was found at R_f value 0.88 for petroleum ether and ethyl acetate extract while 0.78 for methanol extract [Table 3]. After post-chromatographic derivation with anisaldehyde H2SO4, two spots were obtained for petroleum ether extract with R_f value of 0.69, 0.81; four spots for ethyl acetate extract with R_f value

Table 1: Physicochemical parameters of the leaf of *Celastrus paniculatus*

Parameters	Results
Loss on drying at 105°C (% w/w)	8.64
Total ash value (% w/w)	16.08
Acid-insoluble ash (% w/w)	0.386
Water-soluble extractive (% w/w)	14.22
Alcohol-soluble extractive (% w/w)	9.91
Chloroform-soluble extractive (%w/w)	7.75
Ether-soluble extractive (% w/w)	4.74
pH value (5% aqua solution v/w)	5.5

Table 2: Preliminary phytochemical screening of the leaf of *Celastrus paniculatus*

Parameters	Results		
	Petroleum ether extract	Ethyl acetate extract	Methanol extract
Carbohydrate	-	-	+
Proteins	-	-	-
Amino acids	-	-	-
Alkaloids	-	-	+
Tannins	-	-	+
Steroids	+	+	+
Terpenoid	+	+	+
Volatile oil	-	-	-
Glycosides	-	-	+
Fixed oil	+	+	+

(+) indicates presence and (-) indicates absence of that chemical constituent in the plant sample

0.39, 0.54, 0.69, 0.80, and three spots for methanol extract with R_f value 1.42, 0.1, 0.80 [Table 3].

UV spectral comparison for three different extracts was carried out and the densitometric analysis viewed on the

basis of *in situ* evaluation. All three extracts exhibited R_f values of 0.54, 0.69, and 0.80 which are similar in all three tracks [Figures 4-6].
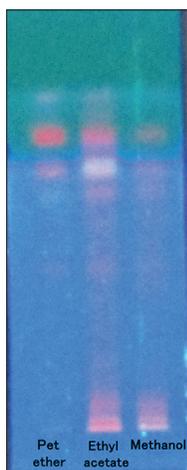

Figure 1: Spots under UV 254 nm

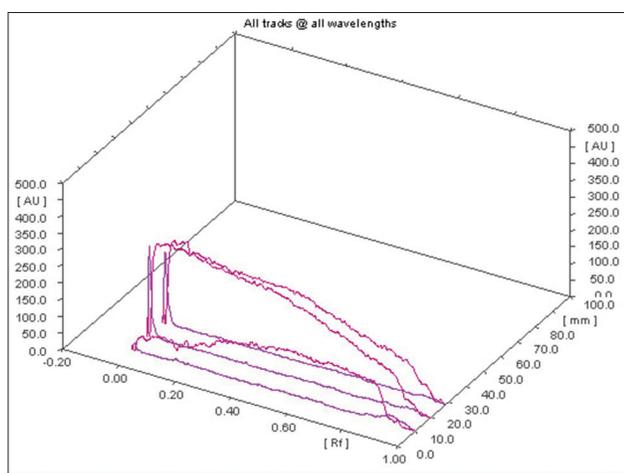**Figure 2: Visible spots after spraying with anisaldehyde-H$_2$SO$_4$ reagent**

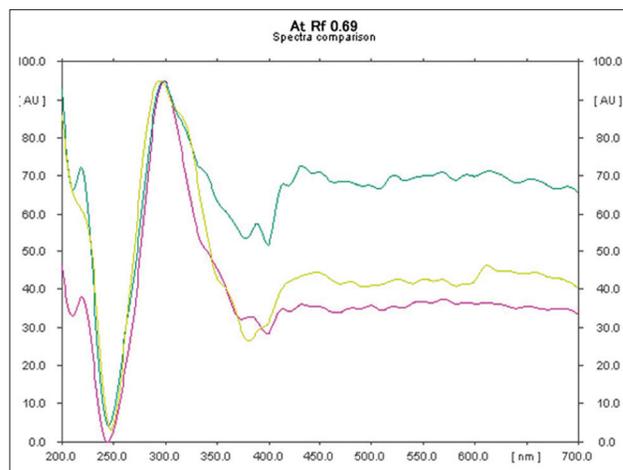**Figure 3: Multiple tracts of all extracts under both 254 nm and 366 nm**

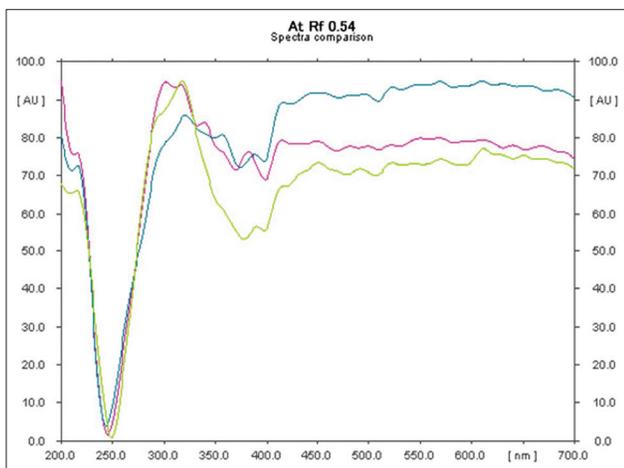**Figure 4: At R_f 0.69 the spectral comparison under 254 nm for the three types of extracts**

Figure 5: At R_f 0.54 the spectral comparison under 254 nm for the three types of extracts
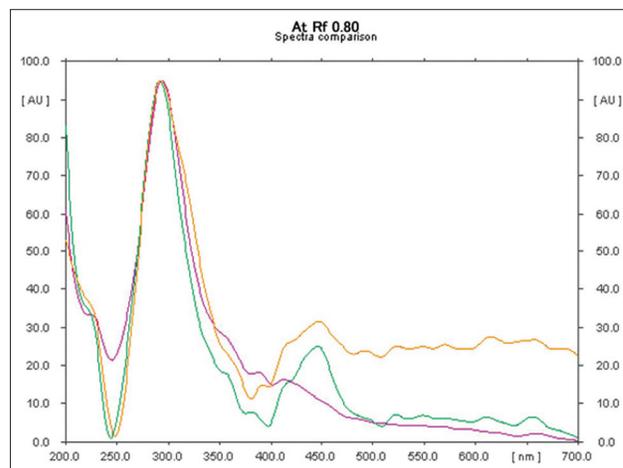

Figure 6: At R_f 0.80 the spectral comparison under 254 nm for the three types of extracts

Table 3: HPTLC profile of Petroleum ether, Ethyl acetate and Methanol extract of leaves of *C. paniculatus*

Sample	254 nm		366 nm		After spraying	
	No. of spots	R_f	No. of spots	R_f	No. of spots	R_f
Petroleum ether extract	12	0.02,0.09,0.13,0.18, 0.24,0.3,0.41,0.49, 0.53,0.69,0.84,0.94	22	0.01,0.05,0.09,0.15, 0.18,0.2,0.22, 0.28,0.35,0.47,0.5,0.55,0.57,0.59,0.71,0.75,0.78,0.81, 0.83,0.88,0.94,0.97	2	0.69,081
Ethyl acetate extract	12	0.02,0.06,0.07,0.12,0.15,0.19,0.39,0.42,0.54,0.69,0.8,0.92	20	0.01,0.08,0.11,0.39, 0.45,0.47,0.5, 0.55,0.59,0.62,0.68,0.72,0.78, 0.79, 0.81,0.84,0.88,0.9,0.94,0.98	4	0.38,0.54,0.69,0.8
Methanol extract	17	0.03,0.07,0.15,0.21,0.42, 0.54,0.580.64,0.67,0.71, 0.78,0.8, 0.83,0.86,0.93,0.98	20	0.01,0.05,0.08,0.11,0.14,0.26,0.27, 0.32,0.42,0.45,0.51,0.61,0.66,0.71, 0.72,0.78,0.8,0.83,0.89,0.95	3	0.42,0.71,0.8

HPTLC: High performance thin layer chromatography

Conclusion

Physico-chemical analysis of the leaves of *C. paniculatus* shows loss on drying 13.05% w/w, total ash value 16.08% w/w, acid insoluble ash 0.386% w/w, water-soluble extractive 14.22% w/w, alcohol-soluble extractive 9.91% w/w. Preliminary phytochemical screening of the petroleum ether and ethyl acetate extracts reveals the presence of steroid and terpenoid while methanol extracts show positive results for steroid, terpenoid, carbohydrate, alkaloid, saponin, and phenolic compounds. HPTLC results have shown specific R_f values for all extracts and these extracts show similar prominent spots at R_f values of 0.54, 0.69, and 0.80 under 254 nm wavelength. These findings may help to generate qualitative and quantitative standards to determine the quality and purity of the plant materials.

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हिन्दी सारांश

विभिन्न ज्योतिष्मती पत्रों का विश्लेषणात्मक अध्ययन

मनोजीत देबनाथ, मौलिशा विश्वास, विनय जे. शुक्ला, के. निष्ठेश्वर

ज्योतिष्मती वैदिक काल से औषधी के रुप मे प्रयुक्त एक काष्ठमय लता है । इस के बीजों से प्राप्त तेल का उपयोग शास्त्र मे वर्णित है । बीजों के विषय में विश्लेषणात्मक अध्ययन हो चुका है । ज्योतिष्मती पत्र के विभिन्न नमुने लेकर उसका विश्लेषणात्मक अध्ययन वर्तमान अनुसन्धान मे किया गया है और एच.पी.टी.एल.सी. आधार पर इनका तुलनात्मक अध्ययन किया गया है ।