

## Research Article

### TLC, HPTLC Studies and Qualitative Phytochemical Investigations of Seeds of *Celastrus Paniculatus* Willd

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#### ABSTRACT

Phytochemical and Pharmacological studies on *Celastrus paniculatus* Willd are an interesting field for research due to its possible medicinal values and the importance of the chemical constituents in the similar variety plants. Thus it makes a great significance to investigate the chemical constituents and study the pharmacological activity on this plant for its medicinal uses, which will be very useful in the field of medicine as a new emerging drug.

Alkaloids, Tannins, Saponins, Steroid, Terpenoid, Flavonoids, and Glycoside distribution in medicinal plant belonging to family was assessed and was investigated. The plant was found to contain Alkaloids, Tannins and Flavonoids, Steroids etc. The significance of the plant in traditional medicine and the importance of the distribution of these chemical constituents in this plant was objective of this study.

**Key-words:** Medicinal Plants, Phytochemical Constituents, *Celastrus Paniculatus* Willd.

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## INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds [1]. Many of these indigenous medicinal plants are used as spices and food plants. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes [2, 3]. Some of the medicinal plants in Tamilnadu, India such as *Tridax procumbens* Linn, *Wedelia chinensis* and *Plectranthus amboinicus* were also studied on the basis of phytochemical constituent's analysis and antimicrobial activity [4]. Chemical profile of *Tridax procumbens* Linn was also investigated [5]. Phytochemical constituents of some Nigerian medicinal plants were also investigated [6]. This study investigates the fundamental scientific bases for the use of medicinal plant by defining and qualitative evaluation of crude phytochemical constituents present in this plant.

## Introduction to plant



**Plant:** *Celastrus paniculatus* willd.

**Family:** *Celastraceae*

**Classical Names:** Jyotishmati, Katabhi, jyotishka, Kangunika, pinya, Lata, Kakaundani, Kakandaki, Katavika, Vega and peetataila.

## Plant Taxonomy

- **Kingdom** : Plantae
- **(Unranked)** : Angiosperms
- **(Unranked)** : Eudicots
- **Order** : Celastrales
- **Family** : Celastraceae
- **Subfamily** : Celastroideae
- **Genus** : Celastrus

**Flowering** - February to April.

**Fruiting** - May to December.

**Altitude** - Plains to 1300m

**Parts Used** - Root, bark, leaf, seed and seed oil.

## Vernacular Name

The various vernacular name of the plant as follow:

- |                 |   |                      |
|-----------------|---|----------------------|
| <b>English</b>  | : | Climbing staff plant |
| <b>Hindi</b>    | : | Malkangni            |
| <b>Bengali</b>  | : | Kondgaidh            |
| <b>Gujarati</b> | : | Malkangana           |
| <b>Marathi</b>  | : | Malkangoni           |
| <b>Punjabi</b>  | : | Sankhu               |

Tamil : Valuluvai  
Telugu : Gundumida  
Oriya : Korsana  
(Ayurvedic Pharmacopeia of India Part-1, 2003)

#### Ayurvedic Properties

RASA : Katu, Tik  
GUNA : Tikshna  
VEERA : Ushna  
VIPAKA : Katu  
PRABHAVA : Medhya

#### MATERIALS AND METHODS

##### Plant material

The fresh seeds of *Celastrus paniculatus* willd were collected during the month of September 2009 from the local market in Jhansi (U.P.). The plant material was identified and authenticated by Dr. H.B. Singh, Scientist F and Head, National Institute of Science Communication and Information of Resources (NISCAIR) New Delhi, with reference number NISCAIR/ RHMD/consult/-2009-10/1291/94.

The seeds of *Celastrus paniculatus* willd were shade dried, and then these are made into coarsely powdered form using dry grinder. The powder plant material was then passed through sieve# 40 & stored in an air tight container for further use. The powdered seeds of the plant (900gm) was packed in Soxhlet apparatus and continuously extracted with petroleum ether(40-60°C) till complete extraction, after completion of extraction the solvent was removed by distillation and then concentrated extract obtained was dried under reduced pressure using rotatory evaporator at temperature not exceeding 40°C. The different extract from each solvent was collected separately in the successive extraction method. The collected each extract was filtered, concentrated and dried using vacuum drier under reduced pressure and dried extract were collected, weighed, and labeled [7].

##### Phytochemical screening

The preliminary phytochemical studies are done for detection of various constituents i.e. alkaloids, glycosides, carbohydrates etc. present in plant extract, which is responsible for the pharmacological activity. Chemical tests were carried out on the successive extracts separately using standard procedures to identify the constituents as described by Sofowara [8], Trease and Evans [9], Harborne [10] and Khandelwal [11].

##### Test for carbohydrates

###### Molish's test (General test)

To 2-3ml. aqueous extract, added few drops of alpha-naphthol solution in alcohol shaken and added conc. H<sub>2</sub>SO<sub>4</sub> from sides of the test tube. Observed for violet ring was formed at the junction of two liquids.

##### Test for reducing sugars

###### Fehling's test

Mixed 1ml. Fehling's A and 1ml. Fehling's B solutions, boiled for one minute. Added equal volume of test solution. Heated in boiling water bath for 5-10 min. First a yellow, then brick red precipitate was observed.

### **Test for proteins**

#### **(a) Biuret test**

To 3 ml. test solution added 4% NaOH and few drops of 1% CuSO<sub>4</sub> solution. Observed for violet color.

#### **(b) Million's test (for proteins)**

Mixed 3 ml. test solution with 5 ml. Million's reagent. Observed for white precipitate.

### **Test for amino acids**

#### **(a) Ninhydrin test (General test)**

Heated 3 ml. test solution in test tube and 3 drops of 5% Ninhydrin solution was added and boiled in boiling water bath for 10 min. Observed for purple color.

### **Test for glycosides**

#### **(a) Legal's test (Test for cardenoloids)**

To aqueous or alcoholic extract, added 1 ml. pyridine and 1 ml. sodium nitroprusside. Pink to red color was not appeared.

#### **(b) Test for deoxysugars (Keller-killiani test)**

To 2 ml. extract, added glacial acetic acid, one drop of 5% FeCl<sub>3</sub> and conc. H<sub>2</sub>SO<sub>4</sub>. Observed for reddish brown color appeared at junction of the two liquid layers and upper layer appeared bluish green.

### **Test for anthraquinone glycosides**

#### **(a) Borntrager's test for anthraquinone glycosides**

To 3 ml. extract, added dilute H<sub>2</sub>SO<sub>4</sub>. Boiled and filtered. To cold filtrate, added equal volume benzene or chloroform, shake well and separated the organic solvent, added ammonia. Ammoniacal layer was not turned pink or red.

#### **(b) Modified Borntrager's test for C-glycosides**

To 5 ml. extract, added 5 ml. 5% FeCl<sub>3</sub> and 5 ml. dil. HCl in test tube. Heated for 5 min. in boiling water bath. Cooled and added benzene or any organic solvent. Shaked well. Separated organic layer, added equal volume dilute ammonia. Ammoniacal layer was observed for pinkish red color.

### **Test for alkaloids**

Evaporated the aqueous, alcoholic and chloroform extracts separately. To residue, added dilute HCl. Shaked well and filtered. With filtrate, performed following tests:

#### **(a) Dragendorff's test**

To 2-3 ml. filtrate in test tube, added few drops Dragendorff's reagent. Orange brown precipitate was formed.

#### **(b) Mayer's test**

To 2-3 ml. filtrate in test tube with few drops Mayer's reagent was added, precipitate was found.

#### **(c) Hager's test**

To 2-3 ml. filtrate in test tube with few drops Hager's reagent was added, yellow precipitate was found.

#### **(d) Wagner's test**

To 2-3 ml. filtrate in test tube with few drops Wagner's reagent was added, reddish brown precipitate was found.

### **Test for tannins and phenolic compounds**

To 2-3 ml. of aqueous or alcoholic extract, added few drops of following reagents:

**(a) 5% FeCl<sub>3</sub> solution:** Deep blue-black color.

**(b) Lead acetate solution:** White ppt.

### **Test for lipids/fats**

A small quantity of powdered drug was rubbed on a filter paper and observed for a permanent translucent strain.

### Test for tannins

About 0.5g of each extract was taken in a boiling tube and boiled with 20ml of distilled water and then filtered. Added few drops of 0.1% of ferric chloride was mixed well and allowed to stand for some time and observed for brownish green or blue black colour formation.

### Test for steroid

#### (a) Salkowski Test

To 2ml of extract, added 2ml of chloroform and 2ml of conc.  $H_2SO_4$ . Shaked well. Chloroform layer appeared red and acid layer shown greenish yellow fluorescence.

### Test for flavonoids test

#### (a) Ammonia test

Filter paper strip was dipped into alcoholic extract and ammoniated. Filter paper strip changed its colour to yellow due to presence of flavonoids.

#### (b) Pew test

To small proportion of extract a piece of metallic magnesium / zinc was added followed by two drops of concentrated hydrochloric acid. A brownish colour confirmed the presence of flavonoid.

### Test for terpenoids

To 0.5g of extract, 2ml of chloroform was added and then conc.  $H_2SO_4$  (3ml) was carefully added to form a layer. A reddish-brown colour formation at the interface was noted for the presence of terpenoids.

Table 1: Qualitative tests of successive extracts

S. No.	Chemical test	Petroleum extract	Ethanollic extract
<b>1.</b>	<b>Test for Alkaloids</b>		
	a) Mayer's reagent	–	+
	b) Dragendorff's reagent	–	+
	c) Wagner reagent	–	+
	d) Hager's test	–	+
<b>2.</b>	<b>Test for Carbohydrates</b>		
	a) Molisch's test	–	–
	b) Fehling's Test	–	–
<b>3.</b>	<b>Test for Glycosides</b>		
	a) Keller Killani Test	–	–
	b) Sodium Nitrosopruside test	–	–
	c) Borntrager's test	–	–
<b>4.</b>	<b>Test for Phenolic Compounds and Tannins</b>		
	a) Ferric chloride test	–	+
	b) Lead acetate test	–	+
<b>5.</b>	<b>Test for flavonoids</b>		
	a) Ammonia test	–	+
	b) Pew test for flavonoids	–	+
<b>6.</b>	<b>Test for Proteins and free Amino Acids</b>		
	a) Million's test	–	–
	b) Ninhydrin test	–	–
	c) Biuret test	–	–
	d) Xanthoprotein test	–	–
<b>7.</b>	<b>Test for Steroids</b>		
	a) Salkowski test	–	+
	b) Liebermann Burchard test	–	+
<b>8.</b>	<b>Test for Triterpene</b>	+	+
<b>9.</b>	<b>Test for Fats and Oils</b>	+	–

[Note: + means present and - means not present]

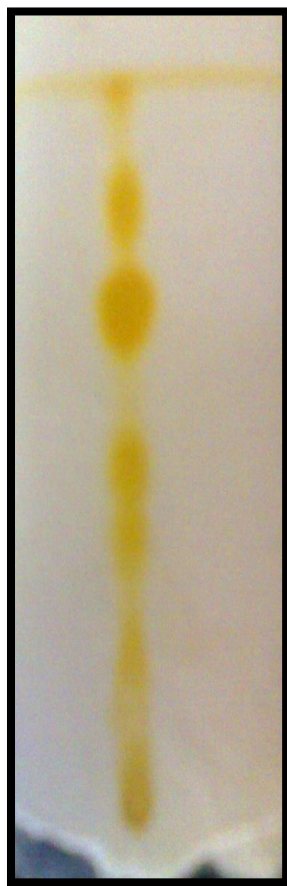
**Thin layer chromatographic study of ethanolic extract**

In 1958, Stahl demonstrated application of TLC in analysis, a method based on adsorption chromatography. The TLC was performed to find out the number of constituents in the respective extract. TLC is the method mainly used to investigate the presence of chemical constituents qualitatively and quantitatively in the plant extract [7, 12, 13]. 100mg of ethanolic extract was weighed and dissolved in 10ml of ethanol and filtered. Filtrate was taken as sample for TLC (Figure 1). According to combination, it was found that Benzene: Chloroform: ethyl acetate (80:10:10) may be the best solvent system. The  $R_F$  values were calculated and results were shown in table 2.

Table 2: TLC of ethanolic extract of *Celastrus Paniculatus Willd.*

S. No.	$R_F$ Values	Colour	Resolution
1.	0.05	Yellow	Good
2.	0.09	Yellow	Good
3.	0.20	Yellow	Best
4.	0.43	Brown	Best
5.	0.58	Brown	Excellent
6.	0.78	Yellow	Excellent
7.	0.95	Yellow	Good

Fig. 1: TLC Chromatogram of Ethanolic Extract of *Celastrus Paniculatus Willd.*



**Adsorbent** - Activated Silicagel G

**Detecting agent** - Iodine vapour

**Solvent system** - Benzene: Chloroform: Ethyl acetate (80: 10: 10)

**HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY**

For the analysis of herbals, HPTLC offers a number of advantages. This technique is especially suitable for comparison of samples on scanning densitometry or video Technology. It has



become a cost and time effective alternative to HPLC. Fingerprint analysis by HPTLC or HPLC is one of the most powerful tools to link the botanical identify to the chemical constituent profile of the plant, in combination with microscopic investigation the fingerprint provides for a convenient identity check.

### HPTLC Parameter

**Sample preparation:** 10mg/ml

**Sample application:** Linomat 5 applicator (Camag)

**Volume applied:** 10 $\mu$ l.

**Solvent system:** Benzene: Chloroform: Ethyl acetate (80:10:10).

**TLC plate development:** Pre saturated Camag Twin Trough Chamber.

### HPTLC Result

HPTLC of extract show the seven peaks, so, the seven peaks give confirmation that the seven compounds may be present in the ethanolic extract of seeds of *Celastrus paniculatus* Willd.

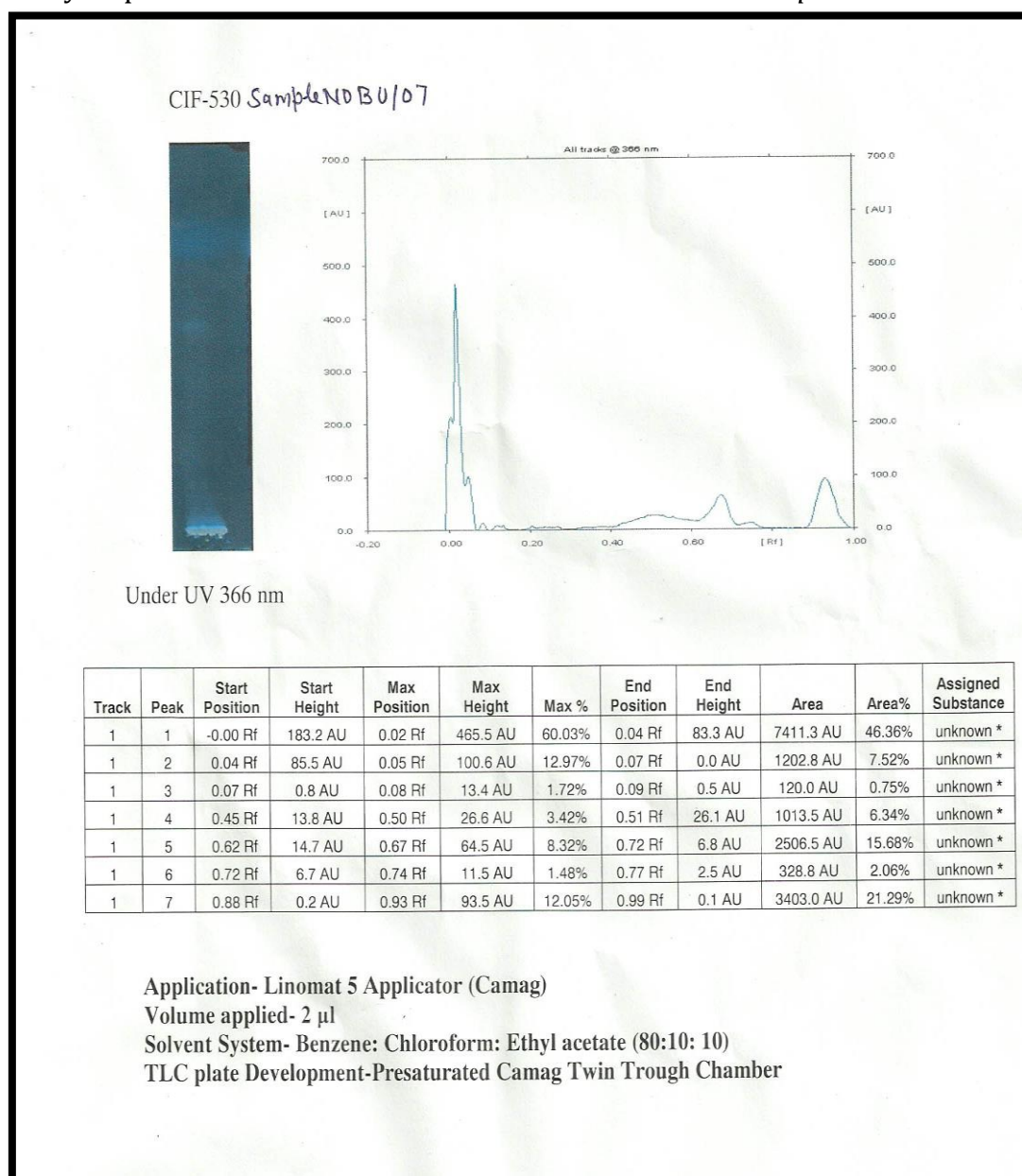


Fig. 2: HPTLC report of ethanolic extract of *Celastrus paniculatus* Willd.

## RESULTS

The present study carried out on the plant samples revealed the presence of medicinally active constituents. The phytochemical characters of the medicinal plant investigated is summarized in Tables 1. Alkaloids, Tannins, Flavonoids, Terpenoids and Glycosides were present in the plant. HPTLC of ethanolic extract was done from NBRI, Lucknow and result found seven peaks (Figure 2) so the seven peaks give confirmation that the seven compounds may be present in the ethanolic extracts of seeds of *Celastrus paniculatus* Willd.

Thus thin layer chromatography studies (Table 2), HPTLC analysis and the qualitative analysis of phytochemical constituents of ethanolic extract of seeds of *Celastrus paniculatus* Willd were concluded as there may be different phytoconstituents in the extract.

## DISCUSSION

The phytochemical screening and chemical constituents of the plant studied showed that the plant was rich in Alkaloids, Flavonoids, Tannins, Terpenoids, Glycosides, Protein and Saponins. They were known to show medicinal activity as well as exhibiting physiological activity [14, 15].

Steroids and tannins were found to be present in the plant. It should be noted that steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones [16, 17]. The presence of terpenoids in this plant can widely used in herbal medicine.

The plant studied here can be seen as a potential source of useful drugs. Further studies are going on this plant in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds. The pharmacological activities of these plants for the treatments of the diseases as claimed by traditional healers are also being investigated [18].

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