



PHARMACOGNOSTIC STUDY AND PHYSICO-CHEMICAL ANALYSIS OF *CELASTRUS PANICULATUS* SEED

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Abstract

The aim of present study was to carry out Pharmacognostic and physicochemical analysis of *Celastrus paniculatus* seeds. (Family; Celastraceae). The present study deals with Pharmacognostic characters as identification parameters of the seeds which were subjected to Macroscopic and Microscopic studies. Phyto-physicochemical studies were done by using WHO recommended parameters and Thin layer Chromatography of the seeds sample was also tested. The microscopy study revealed the presence of calcium oxalate crystals, stone cell, oil globules and aleurone grains etc. Macroscopic study shows orange red crusty aril, a few roughly three sided being convex on the sides and other more or less flat side, surface generally smooth and hard; colour, light to dark brown; odour unpleasant; taste bitter. Physiochemical parameters such as ash values, extractive values, foreign organic matter, TLC analysis were also determined. Preliminary phytochemical screening showed the presence of alkaloids, flavonoids, tannins, sterols, terpenoids. The Phytochemical and Physiochemical analysis of the *Celastrus paniculatus* seed is useful in standardization for quality, purity and sample identification.

Keywords: Pharmacognostic, Physicochemical, Phytochemical, *Celastrus paniculatus*.



Celastrus paniculata wild, Morphology with inflorescence fruit seed and Extract

Synonyms: *Celastrus dependens* wall.

Family: Celastraceae

Plant part used:

Bark, seeds, leaves.

Geographical Distribution:

Plant is distributed throughout the hilly part of Himalaya at an attitude of 1200m, South Gujrat, central India, ceylon, Burma and China.

Botanical Distribution:

It is large deciduous climber with stems upto 23 cm in diameter and 18 m high, sometimes twining dexterously but more often rambling. Twings are fairly smooth, reddish brown, densely covered with small, elongated whitish lenticels. Bark is pale brown, rough with shallow cracks, exfoliating in small soft scales.

Seeds: seeds are completely enclosed in an orange red aril, and are dark brown coloured.

Fruits: fruits are capsular, 1-1.3 cm in diameter, depressed, globose, tri lobed, bright yellow coloured, containing 3-6 seeds.

Medicinal uses:

- Bark is abortifacient.
- Seeds are appetizer, laxative, aphrodisiac, powerful nerve stimulant also used as brain tonic, in treatment of rheumatic pain.
- Seed oil is used as remedy for beriberi & rheumatism.
- Leaves are used as antidote to opium poisoning.
- Flowers have analgesic and anti-inflammatory properties.

Introduction

Celastrus paniculatus plant having remarkable reputation, as a factor of health care and traditionally used in Ayurvedic medicine in India. Plant possesses broad spectrum of activities such as antiviral, antibacterial, insecticidal, analgesic, anti-inflammatory, hypolipidemic, sedative and anticonvulsant also used in rheumatism.

In the present work the seeds of *Celastrus paniculatus* were extracted with different organic solvents namely petroleum ether, hexane, acetone, methanol and respective extracts were phytochemically screened to know the various classes of chemical constituents, and it contains chemical constituents such as sterols, alkaloids, triterpenoids.

Objective of the proposed study is to carry out pharmacogenetic and physicochemical analysis of *Celastrus paniculatus* seed.

Material and Method

A) Collection and authentication of drug:

The seeds were procured from local commercial market of Nagpur, and authenticated by macroscopic and microscopic comparison for their correct botanical identity.

B) Evaluation of raw material (seeds):

Macroscopy:

Dried ripe seeds more or less covered by orange red crusty aril, seeds without aril also present, measuring 5-6 mm in length and 2.5-3.35 mm in breadth, a few roughly three sided being convex on the sides and a few two sides being convex on the sides with one convex and other more or less flat side, one edge of many seeds show a faint ridge or raphe on the whole margin; surface generally smooth and hard; colour, light to dark brown; odour, unpleasant; taste, bitter.

Microscopy:

Show single layered epidermis covered externally with thick cuticle and filled with tannin, followed by 4-6 layers of thin-walled, collapsed, parenchymatous cells and layer of readily elongated stone cells; parenchyma of top one or two layers longer than of the below with triangular intercellular space; inner most layer of parenchyma containing prismatic crystals of calcium oxalate; beneath stone cells layer quadrangular to octagonal, tangentially elongated cells filled with brownish, contents; endosperm composed of polygonal, thin-walled, parenchymatous cells having oil globules and aleurone grains; embryo spatulate in fleshy endosperm containing oil globules and aleurone grains.

Powder:

Oily, dark brown; under microscope shows group of endospermic parenchyma, stone cells, oil globules, aleurone grains and shows fluorescence under U.V. light as following.

Powder as such : Greenish –brown
Powder + 1 NaOH in Methanol : Light green Powder

Foreign organic matter:

Foreign matter is the material consisting of any or all the following.

1. Parts of the organ or organs from which the drug is derived other than the parts named in the definitions and description or for which the limit is prescribed in the individual monograph.
2. Any organ other than those named in the definitions and description.
3. Matter not coming from the source plant.
4. Moulds, insects or other animal contamination.

Method:

The sample seeds (100g) were accurately weighed and spread on tile, inspected with unaided eye and the foreign matter was separated manually. The material thus separated was weighed and the percentage of foreign matter was determined.

a. Total Ash

Crucible was heated for 30 min, then allowed to cool and weighed. Sample (1g) was evenly distributed in the crucible and was allowed to dry at 100^o to 105^oc and ignited to constant mass at 600±25^oc then allowing the crucible to cool after each ignition. Incinerated the residue until the ash was white or nearly so. The percentage of ash was calculated with reference to their air-dried drug.

b. Acid insoluble ash

The ash obtained from the above procedure was boiled with 25 ml of 2M Hydrochloric acid for 5 minutes, filtered through ash less filter paper, transferred to crucible and again ignited, cooled in desiccator and weighed. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

c. Alcohol Soluble Extractive

Powdered sample (5g) was macerated with 100 ml of alcohol in a closed flask for 24 hours, shaking frequently during the first 6 hrs and allowing to stand for 18 hrs. Thereafter, filtered and 25 ml of the filtrate was evaporated to dryness in preterred flat bottom shallow dish on water bath, cooled and weighed. The percentage of alcohol soluble extractive was calculated with reference to the air-dried drug.

d. Water Soluble Extractive

Powdered sample (5g) was macerated with 100 ml of chloroform water in a closed flask for 24 hours, shaking frequently during the first 6 hrs and allowing to stand for 18hrs. Thereafter, filtered and 25 ml of the filtrate was evaporated to dryness in preterred flat bottom shallow dish dried at 105^o, cooled and weighed. The percentage of water-soluble extractive was calculated with reference to the air-dried drug.

Results:Quantitative standards of *Celastrus paniculatus* seeds

| Sr. No. | Parameter | Result (% w/w) | Limit |
|---------|----------------------------|----------------|---------|
| 1 | Foreign organic matter | 1.84 | NMT2% |
| 2 | Total ash | 3.98 | NMT 6% |
| 3 | Acid insoluble ash | 1.05 | NMT1.5% |
| 4 | Alcohol soluble extractive | 44.56 | NLT20% |
| 5 | Water soluble extractive | 10 .14 | NLT9% |

C) Preliminary phytochemical screening of seeds extracts.**Extraction:**

Seeds were air dried in shade under normal environmental condition and then subjected to size reduction to get a coarse powder. Such powdered material was charged into the Soxhlet apparatus and successive extraction was carried out using various solvents such as petroleum ether, hexane, acetone, and methanol. The plants may be considered as a biosynthetic laboratory for a multitude of compounds like alkaloids, glycosides, volatile oils, tannins, saponins, sugars, etc. that exert physiological effect. These compounds (secondary metabolites) are responsible for therapeutic effects. Various extracts were subjected to preliminary phytochemical screening for the detection of various chemical constituents.

Test for sterol:**a. Salkowski Test:**

Extract (2ml) was taken in 2ml of chloroform. Conc. sulphuric acid (2ml) was added from the sides of the test tube. The test tube was shaken for few minutes. If the development of red colour in the chloroform layer indicated the presence of sterols.

b. Liebermann's Test:

Extract (2ml) was taken in a test tube and 2-3 drops of acetic anhydride were added and gently heated. The contents of the test tube were cooled. Few drops of concentrated sulphuric acid were added from the sides of the test tube. If blue colour appear indicated the evidence of presence of sterols.

c. Liebermann's- Burchard Test:

Extract (2ml) was dissolved in chloroform and few drops of acetic anhydride were added to it, followed by concentrated sulphuric acid from the sides of the test tube. If transient colour developed from red blue and finally green then indicated the presence of sterols.

Test for alkaloids:

Extract (2ml) was taken separately in 5 ml of 1.5% v/v hydrochloric acid and filtered. The filtrate was then used for testing alkaloids.

a. Dragendorff's Reagent:

It was prepared by mixing solution A (17 g of bismuth sub nitrate + 200 g of tartaric acid + 800 ml distilled water) and solution B (160 g potassium iodide + 400 ml distilled water) in 1:1 v/v proportion. From this solution, working standard was prepared by taking 50 ml of this solution and adding 100g of tartaric acid and making upto 500ml with distilled water.

The above Dragendorff's reagent was sprayed on Whatmann No. 11 filter paper and the paper was dried. The test filtrate after basification with dilute ammonia was extracted with chloroform and the chloroform. extract was applied on the filter paper, impregnated with Dragendorff's reagent, with the help of a capillary tube. If no orange red colour found on the paper indicated absence of alkaloids.

b. Mayers Reagent (Potassium Mercuric Iodide Reagent):

The Mayer's reagent was prepared as follows:

1.36g of mercuric chloride was dissolved in 60 ml of distilled water. Both the solutions were mixed and diluted to 100 ml distilled water. To a little of the test filtrate, taken in a watch glass, a few drops of the above reagent were added. If no cream-coloured precipitate was formed, indicated the absence of alkaloids.

c. Wagner's Reagent (Iodine- Potassium Iodide):

Iodine (1.27g) and potassium iodide (2g) were dissolved in 5 ml of water and the solution was diluted to 100 ml with water. Few drops of this reagent were added to the test filtrate, if no brown precipitate was formed, indicated the absence of alkaloids.

d. Hanger's Reagent:

A saturated aqueous solution of picric acid was employed for this test. The test filtrate was treated with this reagent. If no precipitate was obtained, indicated the absence of alkaloids.

e. Mayer's Reagent (Potassium Cadmium Iodide):

Potassium iodide (20 g) was dissolved in 20 ml of water and the solution was added to solution containing 10 g of cadmium iodide in 50 ml water. If this reagent with test filtrate gave no precipitate, indicated the absence of alkaloids.

Test of Saponins:**a. Foam Test:**

Extract (2 ml) was taken in a test tube and shaken vigorously with a small amount of sodium bicarbonate and water. If no froth was obtained, indicated the absence of saponins.

b. Haemolysis Test:

Extract (2 ml) was shaken with normal saline. In a series of 5 test tubes, doses of 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1 ml were added and volume was made up to 1ml in each case with normal saline. 1 ml of diluted blood (0.5 ml of rabbit's blood diluted to 25 ml with normal saline) was added to each tube and changes were observed. If no haemolysis of blood occurred, indicated the absence of saponins.

Test of Tannins:

The extract was shaken with water, warmed and filtered. Tests were carried out with the filtrate using following reagents

a. Ferric Chloride Test:

A 5% w/v solution of ferric chloride in 90% alcohol was prepared. Few drops of this solution were added to a little of the above filtrate. If no green color was obtained, indicated the absence of tannins.

b. Lead acetate test:

A 10% solution of basic lead acetate in distilled water was added to the test filtrate. If no precipitate was obtained, indicated the absence of tannin.

c. Potassium Dichromate Test:

On addition of a solution of potassium dichromate in test filtrate. If dark colour was not developed, indicated the absence of tannins.

d) Gelatin solution test:

1% solution of gelatine in water, containing 10% sodium chloride was prepared. A little of this solution was added to a filtrate. If white precipitate was not obtained, indicated the absence of tannins.

Test for flavonoids (Shinoda Test):

A small quantity of test sample was dissolved in 5 ml ethanol (95% w/v) and treated with few drops of concentrated hydrochloric acid and 0.5 g of magnesium metal. If the pink, crimson or magenta colour was developed within few min, indicated the presence of flavonoids.

Test of Coumarines:

Placed a small amount of extract in a test tube and cover the test tube with dilute sodium hydroxide solution. The covered test tube was then placed in a boiling water bath for several minutes, the paper removed and exposed to ultraviolet light. If yellowish green fluorescence appeared within few min, indicated the presence of coumarines.

Test of Proteins:**a. Biuret test:**

Extract (2ml) was vigorously shaken with water and 1ml of 4% of copper sulphate was added to it. If violet or pink colour was not formed, indicated the absence of protein.

b. Xanthoproteic Test:

Extract (2 ml) was shaken with 2 ml of water and 0.5 ml of concentrated nitric acid was added to it. If yellow colour was not obtained, indicated the absence of proteins.

c. Million's Test (Mercury Nitric Solution):

Million's reagent was prepared by dissolving 3 ml of mercury in 27 ml of fuming nitric acid, keeping the mixture well cooled, this solution was then diluted with equal quantity of distilled water. Extract was taken and 2 to 3 ml of Million's reagent was added. If no white precipitate was obtained, indicated the absence of proteins.

Test of Sugars:**a. Molisch's Test:**

The Molisch's reagent was prepared by dissolving 10g α -naphthol in 100 ml of 95% alcohol. Extract (2 ml) was taken in a test tube and mixed with 2 drops of Molisch's reagent. To this solution, 1 ml of concentrated sulphuric acid was added from the side of inclined test tube, so that the acid formed a layer beneath the aqueous solution without mixing with it. If no red brown ring appeared at the common surface of the liquids, indicated the absence of sugar.

b. Barfoed's Test:

This reagent was prepared by dissolving 13.3 g of crystalline neutral copper acetate in 200 ml of 1% acetic acid solution. The test sample was heated with a little of reagent. If no red precipitate was formed, indicated the absence of sugar.

Test For Amino Acids:**a. Ninhydrin Test:**

The Ninhydrin reagent is 0.1% w/v solution of ninhydrin in n-butanol. A little of this reagent was added to the extract. If violet or purple colour was not developed, indicated absence of amino acid.

Test For Fixed Oils:**a. Staining Test:**

Extract when passed against filter paper gave permanent greasy appearance indicated the presence of fixed oils.

b. Sudan Red Test:

Extract (2ml) was taken in a test tube and few drops of Sudan red reagent were added. If red colour obtained, indicated the presence of fixed oils.

TABLE 1- Preliminary phytochemical screening of seeds extracts.

| Sr. No | Chemical constituents | Test / reagent | Petroleum ether extract | Hexane Extract | Acetone extract | Methanol Extract |
|--------|-----------------------|---|-------------------------|----------------|-----------------|------------------|
| 1 | Sterols | 1. Libermann Reaction 2. Libermann Burchard Reaction | + | + | - | - |
| 2 | Terpenoids | 1. Salkowski reaction | + | + | + | + |
| 3 | Alkaloids | 1. Dragendorff's test 2. Wagner's test 3. Hager's test 4. Mayer's test | + | + | - | - |
| 4 | Glycosides | 1. Keller-killiani test 2. Legal's test 3. Baljet test | - | - | - | - |
| 5 | Flavonoids | Shinoda test | - | - | + | + |
| 6 | Saponin | Foam test | - | - | - | - |
| 7 | Tannins | Ferric chloride test | - | - | - | - |
| 8 | Proteins | Lead acetate test Biuret test Million's test | - | - | - | - |

Thin Layer Chromatography:

The extracts were subjected to thin layer chromatography to find out the number of compounds present.

A. Preparation of the plates:

The adsorbent used for the thin layer chromatography was silica gel G. About 25 g of silica gel G was taken in a glass mortar and 35 ml of distilled water was added to it. The mixture was stirred with a glass rod until it became homogenous. The mixture was allowed to swell for 15 min. Then an additional 15 ml of distilled water was added to it with stirring. The suspension was then transferred to a 150 ml flask fitted with a plastic stopper, and was shaken vigorously for about 2 min. Suspension, thus obtained, was then spread immediately on thin layer chromatographic plates.

B. Drying and storage of plates:

The freshly coated plates were then air dried until the transparency of the layer had disappeared. The plates, stacked in a drying rack, were then heated in an oven for 30 min at 110⁰. The activated plates were then kept in desiccator, till required for further use.

C. Application of the sample:

For applying test samples on thin layer chromatographic plates, glass capillaries were used. The spots were applied with the help of fine capillary keeping a minimum distance of 1cm between two adjacent spots. The spots of the sample were marked on the top of the plate to know their identity.

D. Chromatographic chamber, conditions of saturation and the development of TLC plates:

A chromatographic rectangular glass chamber (16.5× 29.5) was used in the experiments. To avoid insufficient saturation and the undesirable edge effect, a smoother chamber in a U shape was allowed and was soaked in the developing solvent. After being thus moistened, the paper was then pressed against the wall of the chamber, so that it adheres to the walls. The chamber was allowed to saturate for 24 h before use. The experiments were carried out at a room temperature in day light.

E. Spraying equipment:

Compressed air sprayer with a fine nozzle was used to detect the different constituents present on TLC plates. Air compressor was attached to the glass sprayer. The sprayer was filled with 50 ml of detection reagent and then used. After each spray the sprayer was washed separately with water, chromic acid, and distilled water and then with acetone.

F. Detection of the spot/s:

Spots were detected using UV light (UV Chamber), iodine vapour and spraying Vanillin- sulphuric acid.

TABLE 2- Thin layer chromatography of different extracts

| Sr No | Sample spot applied | Solvent system used | No. of spots with different locating agents | | | Rf value |
|-------|-------------------------|--|---|--------|--------------------------|------------------------------------|
| | | | UV | Iodine | Vanilline sulphuric acid | |
| 1. | Petroleum Ether Extract | Benzene: Chloroform : Methanol (7:2:1) | -- | 2 | 2 | 0.36 0.66 |
| 2 | Hexane Extract | Benzene: Methanol (9:1) | -- | 2 | 2 | 0.45, 0.69 |
| 3 | Acetone Extract | Hexane : Acetone (8:2) | -- | 3 | 4 | 0.51, 0.62, 0.67, 0.74 |
| 4 | Methanol Extract | Toluene: Ethyl acetate (9:1) | 1 | 3 | 5 | 0:15, 0:20 0:35, 0.54 , 0.63 |

(A) Physical properties of oil:

a) Organoleptic properties:

Colour: Colour was detected by visual inspection by naked eyes.

Odour: Odour was identified by sense organ i.e. inhaling by nasal route.

b) pH: The use of pH to designate the negative logarithm of hydronium ion concentration has proved. In many studies of pharmaceutical interest, it is important to be able to calculate the concentration of all acidic and basic species in solution. The concentration of all species involved in successive acid base equilibrium change with pH and can be represented solely in terms of equilibrium constant and the hydronium ion concentration. The pH of oil was determined by pH meter. The pH meter was calibrated using buffer solution of pH 7.4 and 9.2 and then pH of oil was seen.

c) Viscosity:

Viscosity is an expression of the resistance to flow of a system under an applied stress. Viscosity was determined by Brookfield viscometer.

(B) Analysis Of Oil:

The various physical constants like acid value, acetyl value, saponification value, iodine value etc. were determined by using standard methods of Indian pharmacopoeia.

a. Acid value:

Acid value is the number which expresses in milligrams the amount of potassium hydroxide necessary to neutralize the free acids present in 1g of the substance.

Method

Extract (10g) was accurately weighed and dissolved in 50ml of mixture of equal volumes of ethanol (95%) and ether, which was previously neutralized with 0.1M potassium hydroxide to phenolphthalein solution.

The flask was connected with a reflux condenser and warmed slowly, with frequent shaking, until the sample dissolved. 1 ml of phenolphthalein solution was added and titrated with 0.1 M potassium hydroxide until the solution remained faintly pink after shaking for 30 seconds. The acid value was calculated from the expression

$$\text{Acid value} = 5.61 \frac{n}{w}$$

Where, n = number of ml of KOH

w = weight in gram of sample.

b. Saponification value:

The Saponification value is the number of milligrams of potassium hydroxide necessary to neutralize the free acids and to saponify the esters present in 1 g of the substance.

Method

Accurately weighed 2g of the extract was added in 200 ml flask of borosilicate glass fitted with reflux condenser. Added 25 ml of 0.5 M ethanolic potassium hydroxide and little pumice powder and boiled under reflux on water bath for 30 minutes. It was cooled and then 1ml of phenolphthalein solution was added and titrated with 0.5 M hydrochloric acid (a ml) and repeated the same procedure for blank (b ml).

$$\text{Saponification value} = 28.05 \frac{(b-a)}{w}$$

Where, w = weight in gram of sample

c. Ester Value:

The ester value is the number of milligrams of potassium hydroxide required to saponify the esters present in 1g of the substance.

Method

Acid value and saponification value were determined as per the pharmacopoeial procedures and the ester value was calculated from the expression.

$$\text{Ester value} = \text{saponification value} - \text{acid value}$$

d. Hydroxyl value:

The hydroxyl value is the number of milligrams of potassium hydroxide required to neutralize the acid combined by acylation in 1g of the substance.

Method

50 ml acylation flask fitted with a reflux condenser, add 1 g oil, 12 g stearic anhydride and 10 ml xylene, heat under reflux for 30 minutes. Cool, add a mixture of 40 ml pyridine and 4ml water, heat for further 30 minutes and titrate the hot solution with 1M potassium hydroxide solution using phenolphthalein indicator and same procedure was followed for blank.

$$\text{Hydroxyl value} = 56.11 \frac{v}{w}$$

Where,

V = difference in ml, between the titrations,

W = weight in gram of substance.

e. Iodine value: The iodine value is a number which expresses in grams the quantity of halogen, calculated as iodine, which is absorbed by 100 g of substance under the described conditions.

Method (Iodine Monochloride Method or Wijs Method)

In a dry 500 ml iodine flask, accurately weighed (0.200g) oil was taken and to it 10 ml carbon tetrachloride was added and dissolved. Iodine monochloride solution (20 ml) was added and stopper was inserted. It was allowed to stand in dark at a temperature between 150⁰ and 250⁰ for 30 minutes. Then 15 ml of potassium iodide solution was added in cup top by side of flask, rinsed with 100 ml of water, shake and titrated with 0.1M sodium thiousulphate using starch solution as indicator, added towards the end of the titration. Noted the number of ml. required (a ml) and repeated the same procedure omitting the substance being examined and note the ml required (b ml)

$$\text{Iodine value} = 1.269 \frac{9(b-a)}{w}$$

TABLE 3- Physical Parameters

| Sr. no. | Physical parameter | Result |
|---------|--------------------|--|
| 1. | Colour Odour | Brownish red Pungent |
| 2. | Solubility | Chloroform, toluene, diethyl ether, methanol n-hexane, acetone |
| 3. | pH | 7-7.1 |
| 4. | Viscosity | 16.6-19.6 |
| 5. | Refractive index | 1.51 |

Table 4

| Sr. no. | Chemical parameter | Value |
|---------|----------------------|--------|
| a. | Acid value | 11.93 |
| b. | Saponification value | 238.13 |
| c. | Ester value | 220.2 |
| d. | Hydroxyl value | 308.6 |
| e. | Iodine value | 104.46 |

Conclusion:

Literature survey reveals that the plant possesses a broad spectrum of biological activities. The result of phytochemical screening reveals that major constituents of oil were triterpenoids, alkaloids and sterols. So in future, attempt should be made to isolate the active constituents from the extracts by using separation technique such as column chromatography and incorporate them in a better developed formulation.

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