

The Phytochemical Analysis and Anthelmintic Potential of *Celastrus paniculatus* Seed: An *In-Vitro* Assessment

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Abstract

As the quality requirements are predicated on appropriate raw material selection, standardization is important to the development of phytopharmaceuticals of standard quality. A traditional Ayurvedic medicine plant, *Celastrus paniculatus* Wild (Celastraceae), has been used for millennia as a sedative, antiepileptic, analgesic, anti-inflammatory, and memory-enhancing drug. For rheumatism, gout, paralysis, and leprosy, a decoction of seeds is prescribed. The current study looks at morphological and microscopic characteristics, ash value, extractive values, and phytochemical assessments, which include a qualitative chemical analysis of the active ingredients. It was found that different seed extracts had varying degrees of anthelmintics.

Keywords: *Celastrus Paniculatus Seed*, Phytochemical, Phytoconstituent, Pharmacognostic & Anthelmintics Efficacy.

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INTRODUCTION

Since appropriate raw material selection is the foundation of quality standards, standardization is essential to the manufacturing of phytopharmaceuticals of standard quality. Because the official monographs mention very few particular standards, the pharmaceutical business stands to lose greatly from the examination of crude medications. This includes figuring out who you are and how pure your product is. The purity of any crude medicine is impacted by several organic and inorganic impurities that are nearly hard to avoid when gathering crude drugs. As a result, appropriate

assessment and detection based on various pharmacognostic & phytochemical parameters is required [1]. A key component of all traditional medical systems is a herbal remedy. In accordance with Ayurvedic and other ancient medical systems, some 1250 medicinal plants are employed in India to create therapeutic formulations [2]. Since ancient times, *Celastrus paniculatus* Wild, referred to in Ayurveda as the "Tree of Life" (Celastraceae), has been used to cure brain problems and improve memory and learning. In addition to its primary activity—the memory-enhancing effect—*C. paniculatus* demonstrated a variety of other actions.



Antiviral, antibacterial, insecticidal, anti-inflammatory, antispermatogenic, sedative, analgesic, anti-fatigue, and hipolipidemic are only a few of the

documented activities. It has antirhumatic, aphrodisiac, arthralagenic, emetic, laxative, and nervine tonic properties [3, 4].

An illness caused by helminths that stops living things from growing normally is called helminthiasis, and it is quite dangerous. Helminthiasis, an intestinal infection, is the most common infectious disease in the developing world. Apart from their exorbitant cost, helminths are currently resistant to drugs that are sold commercially. Diseases caused by parasites still significantly reduce the number of live animals produced worldwide. Feeding on the blood of small ruminants, the parasitic worm *Haemonchus contortus* causes anemia, appetite loss, sluggish growth, and ultimately death in its host. The highly pathogenic parasite of small ruminants, *H. contortus*, severely hinders the global production of healthy sheep and goats [5]. Using anthelmintic medications derived from organic plant sources, researchers are attempting to address the problems.

MATERIALS AND METHODS

Collection and Authentication: Seed of *Celastrus paniculatus* seed were collected from local market of Lucknow (U.P.) and authenticated.

Preparation of Plant Extracts

800 ml of petroleum ether (40–60°C) was used to extract about 200 g of dried powdered *Celastrus paniculatus* seeds at a temperature of 40–50°C. The solvent in the thimble was extracted until it turned clear. The extract was then concentrated over a water bath until an oily extract was obtained, after which the solvent was distilled out.

Preliminary Phytochemical Screening of Plant Extracts [6- 8]

Characterization of Seed oil of *Celastrus paniculatus* Acid Value

A precise transfer method was used to weigh 2 g of oil into a 250 ml conical flask. Using a pipette, 20 milliliters of neutral ethanol were added, and the flask was heated for three minutes in a steam bath. After that, the flask was allowed to cool, and phenolphthalein was used as an indicator to titrate the contents using a 0.1M alcoholic KOH solution. Additionally, a blank titration was carried out side by side.

Iodine Value

Using the transfer method, 2 g of oil were precisely weighed into a 250 ml iodine flask and dissolved in 20 ml of chloroform. With a pipette, 20 ml of Wij's reagent (iodine monochloride) was added. The stopper-equipped flask was left in the dark for an hour while being shaken periodically. After adding 10 milliliters of 15% potassium iodide solution and 50 milliliters of distilled water to the flask, the mixture was thoroughly shaken. Using fresh starch solution as an indication, the released iodine was titrated with 0.1M sodium thiosulphate solution. Additionally, a blank titration was carried out side by side.

Saponification Value

Via the transfer method, 2 g of oil were precisely weighed and placed into a 250 ml round-bottom flask. Using a pipette, add freshly made 0.5M alcoholic potassium hydroxide solution (25 ml) to the sample. Gently reflux the mixture over a steam bath with an air conditioner for one hour. After cooling the flask to a temperature between 60 and 70°C, the condenser tip was cleaned with a little amount of distilled water, and the contents were titrated using a 0.5M HCl solution with phenolphthalein acting as an indicator. Concurrently, a blank titration was performed.

Preliminary Qualitative Test

A preliminary qualitative phytochemical analysis was conducted on the different extracts of *Celastrus paniculatus* seed. The list below includes the different tests and reagents utilized.

Alkaloids

Preparation of test solution: The extracts were dissolved in the diluted hydrochloric acid to create the test solution.

Mayer' Test: The acidic test solution with Mayer's reagent (Potassium Mercuric iodide) gave cream colored precipitate.

Hager's Test: The acidic test solution with Hager's reagent (Saturated picric acid solution) gave yellow precipitate.

Dragendorff's Test: The acidic solution with Dragendorff's reagent (Potassium bismuth iodide) showed reddish brown precipitate.

Wagner's Test: The acidic test solution treated with Wagner's reagent (Iodine in potassium iodide) gave brown precipitate.

Tannic acid Test: The acidic test solution treated with Tannic acid gave buff colour precipitate.

Picrolonic Acid Test: Alkaloids gave yellow colour precipitate with picrolonic acid.

Amino Acid:

Millon'test: To the test solution add about 2 ml of millon's reagent white precipitate indicates presence of amino acid.

Ninhydrine Test: To the test solution add Ninhydrine solution, boil, violet colour indicates presence of amino acid.

Carbohydrates

Preparation of Test Solution:

The test solution was made by dissolving the test extracts in water. Following hydrolysis in one

volume of N-HCL, it was subjected to the next chemical test.

Molisch's Test:

Test solution with two milliliters of conc. H₂SO₄ slowly added from the test tube walls, along with a few drops of Molisch's reagent. It displayed a purple ring where two liquids came together.

Barfoed's Test:

If red cupric oxide forms after heating 1 milliliter of the test solution with 1 milliliter of Barfoed reagent on a water bath, monosaccharide is present. Because disaccharides partially hydrolyze to monosaccharide after prolonged heating (about 10 minutes), they may also result in decrease.

Benedict's Test: Test solution treated with Benedict's reagent and after boiling on water bath, it showed reddish brown precipitate.

Fehling's Test:

The test solution when heated with equal volume of Fehling's A and B solution, gave orange red precipitate, indicating the presence of reducing sugars

Phenols

The parent material known as flavones is the structural source of all flavonoids. Both free form and bonded sugars as glycosides include flavonoids. Because of this, it is usually preferable to analyze the flavonoids found in hydrolyzed plant extracts when conducting flavonoid analysis.

Preparation of Test Solution:

A test tube was filled with a tiny amount of extract, equal volume of 2 M HCL, and heated to 1000C for 30 to 40 minutes. After cooling, the extract was filtered and ethyl acetate was added. To check for flavonoids, the ethyl acetate was concentrated until it was completely dry.

Shinoda Test:

The color of the test solution turned pink to magenta red when a few pieces of magnesium ribbon and conc. HCL were added. When lead acetate solution was added to a little amount of test solution, a yellow-colored precipitate appeared.

Alkaline Reagent Test:

The test solution's yellow color intensity increased when it was treated with sodium hydroxide solution; however, this color intensity decreased when a few drops of diluted acid were added.

Glycosides

Preparation of Test Solution: The test solution was prepared by dissolving extract in the alcohol or hydro-alcoholic solution.

Test for Cardiac Glycosides:

Kedde' Test:

To 90% alcohol, add one drop and two drops of 2% 3, 5-dinitro benzoic acid. Alkalize with 20% sodium hydroxide solution to obtain a purple color. In order for 3, 5-dinitro benzoic acid to cause a color reaction, the aglycone must contain α , β -unsaturated lactones.

Baljet's Test: The test solution treated with sodium picrate gave yellow to orange colour.

Raymond's Test: Test solution treated with hot methanolic alkali, violet colour is produced.

Bromine Water Test: Test solution dissolve in bromine water give yellow precipitate.

Keller-Killani Test for Digitoxose:

The test solution treated with few drops of FeCl₃ solution and mixed, then H₂SO₄ containing FeCl₃ solution was added, it formed two layers. Lower layer reddish brown, upper layer turns bluish green.

Legal's Test: Test solution when treated with pyridine (made alkaline by adding sodium nitroprusside solution) gave pink to red colour.

Test for Anthraquinone Glycosides:

Borntrager's Test:

Drug powder was boiled for five minutes with five milliliters of 10% sulfuric acid. Hot filtering was followed by a cooling process and a gentle shaking with an equivalent volume of benzene. After the benzoene layer was separated, ammonia (10%) was added at half of its content. Permitted to divide it. Because anthraquinones are present, the ammonical layer developed a rose pink color.

Proteins

Preparation of test solution: The test solution was prepared by dissolving the extract in water.

Millon's Test: Test solution was treated with millon's reagent and heated on a water bath. The proteins were stained red

Biuret Test: Test solution was treated with 40% sodium hydroxide and dilute copper sulphate solution gave blue colour.

Xanthoproteic Test: Test solution was treated with conc. HNO₃ and boiled which gave yellow precipitate.

Modified Borntrager's Test:

The hydrolysis of anthraquinone C-glycosides requires more severe conditions. Using 5 ml of diluted HCL and 5 ml of 5% FeCl₃ solution, the medication was hydrolyzed. The process for the hydrolyzed extract was completed in accordance with Borntrager's test guidelines.

Test for Steroids**Preparation of Test Extract Solution:**

The extract was refluxed independently with a potassium hydroxide alcoholic solution until full saponification. Diethyl ether was used to remove unsaponifiable materials from the saponified extract after it had been diluted with water. After the ethereal extract was evaporated, the residue—also known as saponifiable matter—was dissolved in chloroform and used as a test sample.

Salkowski Test:

A few drops of concentrated H₂SO₄ were added to the test extract solution, shaken, and left to stand. The lower layer turned crimson, signifying the presence of steroids.

Libermann - Burchard Test:

After adding a few drops of acetic anhydride to the test solution and mixing it, the upper layers turned green and a brown ring appeared at the intersection of the two levels when concentrated H₂SO₄ was added from the test tube walls. A small amount of concentrated H₂SO₄ was added. The color blue emerged.

Sulphur Test: Sulphur test when added in to the test solution, it sank it.

Tannins and Phenol Compound

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

5% FeCl₃ solution: Deep blue- black colour.

Lead Acetate Solution: White precipitate.

Bromine Water: Discoloration of bromine water

Acetic Acid Solution: Red colour solution.

Triterpenoids

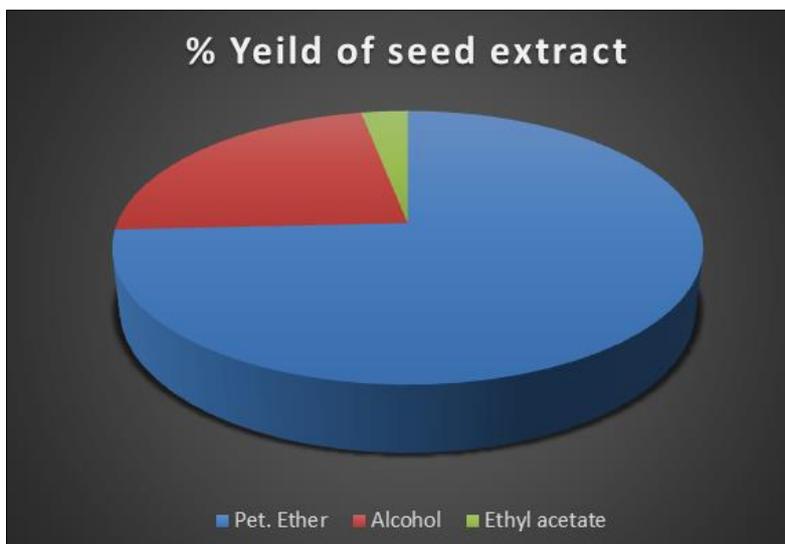
Preparation of Test Extract Solution: The test extract solution was prepared by dissolving extract in the chloroform.

Salkowski Test:

Few drops of concentrated sulphuric acid were added to the test solution, shaken and on standing lower layer turned golden yellow [8-10].

Table No 1: Percent yield and physical evaluation of extracts

Sr. No.	Extract	Nature of Extract	Colour	Weight	% Yield
1.	Pet. Ether (40-60 ⁰)	Liquid (Oil)	Reddish Brown	160 g	32.33% w/w
2.	Alcohol	Semi solid	Brownish Black	49 g	27.85% w/w
3.	Ethyl acetate fraction		Dark brown	6.52g	20.66% w/w

**Fig. 1: % yield of various seed extract *Celastrus paniculatus*****Table No2: Physiochemical parameter *Celastrus paniculatus* seeds**

Oil Characteristic	Result
Acid value	3.54
Saponification value	218.41
Ester value	214.83
Iodine value	103.16
Unsaponifiable matter	0.1950

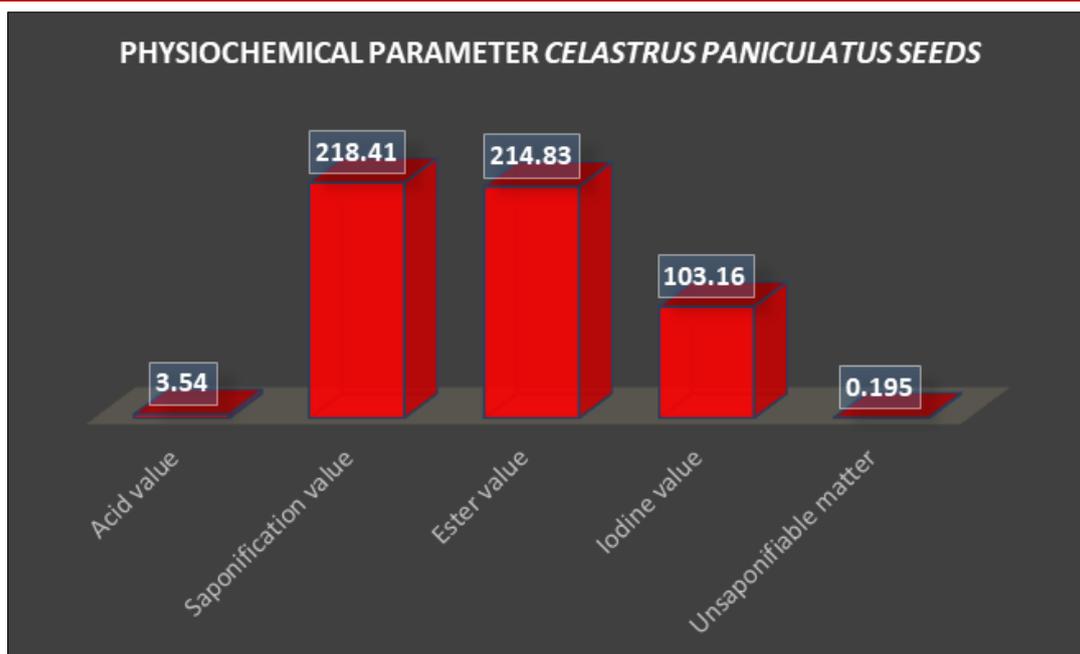


Fig. 2: Physiochemical parameter *Celastrus paniculatus* seeds

Table No 3: Phytochemical analysis of different extract of *Celastrus paniculatus* seeds DC

Sl. No.	Phytoconstituent	Pet. Ether (40-60°C) Extract	Alcoholic extract	Ethyl acetate fraction of Alcoholic extract
1.	Carbohydrates	-	-	-
2.	Proteins	-	-	-
3.	Amino acids	-	-	-
4.	Fats and oils	+	+	+
5.	Steroids	+	+	-
6.	Glycosides	-	+	-
7.	Alkaloids	+	+	-
8.	Tannins	-	+	-
9.	Vitamins	-	-	-
10.	Flavonoids	-	+	+
11.	Triterpenoids	+	+	+

Table 4: Effect of seed extract of *Celastrus paniculatus* on paralytic time and death time of *Pheretima posthuma*

Extract	Dose (mg/mL)	Mean length of worms (cm)	Paralytic time (min) Mean \pm SEM	Death time (min) Mean \pm SEM
PEE	10	10	97.1 \pm 4.6*	405 \pm 27.5*
	25	9	82.8 \pm 19.5 *	311 \pm 14.7*
	50	11	23.0 \pm 57.1 *	214 \pm 14.10*
EAE	10	6	29.5 \pm 2.50*	21.2 \pm 2.20
	25	7	11.1 \pm 2.49	10.4 \pm 5.08
	50	6	7.8 \pm 50.1	211 \pm 15.02
EE	10	8	71.2 \pm 2.5*	511 \pm 30.4*
	25	7	20.9 \pm 21.6*	374 \pm 20.2*
	50	9	3.6 \pm 1.35	6.04 \pm 0.96
Albendazole	10	4	3.4 \pm 0.75	6.1 \pm 2.01



Fig. 2: In-vivo Anthelmintic Activity

RESULT AND DISCUSSION

The literature makes clear how significant *Celastrus paniculatus* seeds are due to their numerous therapeutic benefits. Numerous pharmacological properties of the plant are demonstrated, including hipolipidemia, anti-inflammatory, antispermatogenic, sedative, anti-fatigue, and analgesic effects. Several physical constants, including acid value (3.04), iodine value (103.16), saponification value (218.41), ester value (214.83), and unsaponifiable matter (0.1950 g) were measured for the seed oil (Table No.2 & fig 2). Alcohol and pet ether (40–600C) were used to extract the dried powdered seeds of *C. paniculatus*. 32.33% and 27.85% of the extracts were found to have a yield percentage, respectively. Ethyl acetate was used to fractionate the thirty grams of alcoholic extract. According to Table No. 1 & fig 1, the yield percentage of the ethyl acetate fraction was 20.66%w/w. Several phytoconstituents were found in each extract and fraction, according to the preliminary phytochemical analysis (Table No. 3). The findings showed that although alcoholic extract displayed the presence of alkaloids, flavonoids, fats and oil, sterols, and tannins, Pet. ether extract demonstrated the presence of sterols, fats and oil, triterpenoids, and alkaloids. The presence of fats, steroids, and flavonoids was demonstrated by the ethyl acetate fraction. A major health concern is helminthiasis, an illness caused by helminths that results in difficulties and stunted growth in living things. Helminthiasis, an intestinal infection, is the most common infectious disease in the developing world. Nowadays, helminths are resistant to drugs that are sold commercially, and they are highly costly. In an attempt to address the problems, researchers are working to filter the anthelmintic chemicals from natural plant sources. The current focus is on identifying and investigating plants with potential as anthelmintics. The medications used to treat helminthiasis at the moment are expensive, only effective against a single kind of parasite, and lose their effectiveness after 20 minutes. These active ingredients may be responsible for the anthelmintic action of the *C. paniculatus* seed extract. The anthelmintic activity in vivo ascertained in PEE, EAE, and EE. PEE, EAE, and EE of the seed extract of *C. paniculatus* shown anthelmintic properties against *Pheretima posthuma* in our investigation (Table 4 & fig. 2). The outcome showed that, when compared to the

standard, the ethanolic seed extract of *C. paniculatus* exhibited the highest level of activity. The most effective treatment for *Pheretima posthuma* was ethyl acetate extract at a dose of 50 mg/mL. At 3.6 minutes, the extract had a paralyzing effect; at 6.04 minutes, it actually caused death. The effects of EE at 50 mg/mL against *Pheretima posthuma* (earthworm) were not substantially different from those of albendazole at 10 mg/mL, despite the fact that PEE, EAE, and EE showed dose-dependent action.

CONCLUSION

The seeds of *Celastrus paniculatus* have noteworthy antinociceptive and anti-inflammatory properties. In the current study, the anthelmintic potential of many seed extracts was assessed. EE of the *C. paniculatus* seed demonstrated a noteworthy outcome in comparison to the standard. Additional research endeavors could concentrate on bioactivity-guided fractionation and isolation in order to identify potential phytoconstituents accountable for the aforementioned activities.

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