RESEARCH PAPER

Pharmacognostic Standardization, Phytochemical Analysis, and Psychopharmacological Assessment of Celastrus paniculatus

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ABSTRACT

Background: Celastrus paniculatus Willd. (Celastraceae), traditionally classified as a Medhya Rasayana in the Ayurvedic system of medicine, is widely revered for its profound neuroprotective and cognitive-enhancing properties. The present study was meticulously designed to establish comprehensive pharmacognostic standards, evaluate the phytochemical profile via chromatographic fingerprinting, and validate the psychopharmacological efficacy of its extracts.

Material and Methods: The botanical identification and quality control standardization were achieved through macroscopic, microscopic, and physicochemical evaluations. Successive solvent extraction was followed by qualitative phytochemical screening. Following acute oral toxicity testing (OECD Guideline 423), the psychopharmacological potential was assessed in Swiss albino mice using the Forced Swim Test (FST), Elevated Plus Maze (EPM), Hole-board test, and Actophotometer at therapeutic doses of 200 and 400 mg/kg p.o.

Results: Pharmacognostic investigations established species-specific diagnostic markers, including distinct quantitative leaf constants and powder microscopy features. Phytochemical analysis of the methanolic extract revealed a high abundance of flavonoids, triterpenoids, and phenolic compounds, with HPLC successfully quantifying celastrol at 2.45% w/w. In vivo behavioral models demonstrated that the methanolic extract (400 mg/kg) produced highly significant ($p < 0.001$), dose-dependent antidepressant-like effects by reducing immobility time in the FST. Furthermore, it exhibited potent anxiolytic activity, evidenced by increased open-arm exploration in the EPM and enhanced head-dipping frequency in the hole-board paradigm, alongside a moderate, balanced reduction in spontaneous locomotor activity.

Conclusion: The established pharmacognostic parameters and chromatographic fingerprints provide robust analytical tools for the stringent quality control of C. paniculatus. The significant antidepressant and anxiolytic activities experimentally validate its traditional ethnopharmacological claims, strongly suggesting its therapeutic potential in managing neuropsychiatric disorders, largely attributable to bioactive phytoconstituents like celastrol.

Keywords: Celastrus paniculatus, Pharmacognostic standardization, Celastrol, HPTLC fingerprinting, Antidepressant, Anxiolytic activity

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1. Introduction

Herbal medicine has been an indispensable cornerstone of human healthcare since antiquity, providing a vast reservoir of therapeutic agents for the management of numerous acute and chronic ailments. Across various global civilizations, traditional medicinal plants have served as the primary source of healthcare, relying on holistic approaches and synergistic interactions among natural phytoconstituents to restore physiological balance (Balkrishna et al., 2024). In recent decades, the paradigm of modern pharmaceutical drug discovery has increasingly pivoted back to these traditional roots through the multidisciplinary, scientific lens of ethnopharmacology. Ethnopharmacology systematically investigates the biological, anthropological, and pharmacological basis of indigenous medical systems, facilitating the translation of historical folklore remedies into modern, evidence-based therapeutics (Leonti & Casu, 2013). This essential transition is profoundly supported by the contemporary concept of reverse pharmacology, an innovative transdisciplinary approach that integrates documented clinical experiences and experiential observations from traditional medicine into robust preclinical and targeted clinical research (Surh, 2011). By initiating the drug discovery process from well-established traditional formulations and working backward to identify the active biological principles, structural mechanisms of action, and optimal dosage profiles, reverse pharmacology significantly expedites the development of safe, economical, and highly efficacious botanical drugs. Despite the immense therapeutic potential and historical success of herbal medicines, their global regulatory acceptance and widespread clinical integration are frequently hindered by persistent challenges related to adulteration, substitution, and significant batch-to-batch phytochemical variability. To meticulously mitigate these issues, the World Health Organization (WHO) has formulated comprehensive and stringent guidelines emphasizing the rigorous standardization of all herbal drugs to unequivocally ensure their absolute quality, uncompromised safety, and reliable efficacy (Indrayanto, 2024). This standardization process encompasses a multidimensional and systematic evaluation, including macroscopic and microscopic authentication, detailed physicochemical profiling, and the strict determination of permissible safety limits for toxic contaminants such as heavy metals, microbial loads, and pesticide residues. Furthermore, the inherent chemical complexity of diverse plant matrices necessitates highly advanced analytical interventions for proper characterization (Nicoletti, 2011). Such high-resolution, hyphenated techniques provide highly reliable mechanisms to validate the overarching chemical consistency of herbal preparations, ultimately ensuring that subsequent pharmacological and toxicological evaluations are conducted on reproducible, pure, and scientifically well-characterized plant extracts. Among the myriads of potent botanicals extensively documented within the traditional Indian system of medicine, *Celastrus paniculatus* Willd. (belonging to the family Celastraceae), colloquially known in vernacular languages as Malkangni or Jyotishmati, occupies an exceptionally prominent and revered position. Geographically distributed widely across the varied subtropical and tropical regions of the Indian subcontinent, this large, woody climbing shrub is highly regarded in the classical Ayurvedic system of medicine (Shukla et al., 2012). It is classically categorized under the specialized domain of *Medhya Rasayana*, an elite group of rejuvenating therapeutic herbs specifically renowned for their profound nervine tonic, memory-enhancing, and intellect-promoting neuro-pharmacological properties. Traditionally, the processed seeds and the extracted golden-yellow seed oil of *C. paniculatus* have been effectively administered by traditional healers to alleviate severe cognitive deficits, mitigate mental fatigue, and treat a broad spectrum of central nervous system disorders. Contemporary pharmacological investigations have robustly begun to corroborate these ancient traditional claims, scientifically revealing that the plant extracts possess remarkably potent neuroprotective, anxiolytic, antidepressant, and intrinsic antioxidant activities, which are largely attributed to their rich, diverse array of sesquiterpene polyol esters, unique alkaloids, and powerful flavonoids (Shukla et al., 2012). However, a comprehensive, multi-tiered scientific validation that seamlessly bridges its

fundamental pharmacognostic identity, its precise and highly reproducible phytochemical blueprint, and its definitive psychopharmacological mechanisms of action in vivo remains a critical prerequisite for its transition into clinical advancement. Therefore, the present research study is systematically designed to execute a stringent pharmacognostic standardization and a highly detailed psychopharmacological assessment of *Celastrus paniculatus*. By establishing robust, modern quality control parameters and directly correlating the isolated chemical constituents with their corresponding central nervous system activities using scientifically validated behavioral animal models, this research definitively aims to provide a solid, evidence-based pharmacological foundation for the safe and targeted therapeutic application of *C. paniculatus* in modern, integrative neuropsychiatric care.

2. Materials and Methods

2.1. Collection and Identification

Fresh aerial parts and seeds of *Celastrus paniculatus* were collected from their local natural habitat in Uttar Pradesh during their optimal flowering and fruiting season. All plant parts were washed with distilled water, shade-dried at room temperature, and ground into a coarse powder using a mechanical pulverizer. The powdered material was then stored in airtight containers for subsequent experiments (Smith et al., 2021).

2.2. Assessment of Quality of Plant Materials

Pharmacognostic parameters were evaluated in accordance with standard WHO guidelines to establish the quality, purity, and authenticity of the crude drug (World Health Organization, 2011).

2.2.1. Determination of Foreign Matter

To assess the preliminary purity of the sample, 100 g of the dried botanical drug was spread out and observed using the naked eye and a 10x magnifying lens. Any extraneous materials (such as stones, soil, animal excreta, or morphologically similar plant parts) were manually separated and weighed, and the percentage of foreign matter (% w/w) was calculated (Khandelwal, 2008).

2.2.2. Macroscopic Evaluation

A macroscopic and organoleptic examination of the various parts of *C. paniculatus* (leaf, stem, and seed) was performed. Through visual inspection and sensory evaluation, distinct morphological attributes including color, odor, taste, size, shape, and surface texture were meticulously recorded (Evans, 2009).

2.2.3. Microscopic Evaluation

To study the internal tissue architecture, fine transverse sections (T.S.) of the fresh leaf, stem, and root were prepared using a rotary microtome. These sections were first cleared using a saturated chloral hydrate solution and subsequently stained with a mixture of phloroglucinol and concentrated HCl (1:1) to precisely identify lignified structural elements such as xylem vessels and sclerenchyma. For powder microscopy, the dried fine powder was mounted on a glass slide with glycerin and selective stains (safranin/iodine), allowing characteristic diagnostic features like trichomes, starch grains, and calcium oxalate crystals to be observed under a light microscope (Mukherjee, 2019).

2.2.4. Quantitative Microscopy

Leaf surface constants were determined using standard procedures and a calibrated camera lucida/ocular micrometer. The stomatal index, palisade ratio, vein-islet number, and vein-termination number were accurately calculated for both the adaxial and abaxial surfaces of the leaf samples. To ensure statistical reliability, a minimum of 10 distinct microscopic field readings were recorded for each anatomical parameter to derive their mean values (Wallis, 2005).

2.3. Proximate Analysis (Physicochemical Parameters)

The physicochemical parameters of the *Celastrus paniculatus* powder were evaluated according to the standard guidelines of the WHO and the Ayurvedic Pharmacopoeia of India (API) to firmly establish the purity, identity, and strength of the botanical drug (Ayurvedic Pharmacopoeia of India, 2008; World Health Organization, 2011).

2.3.1. Loss on Drying (LOD)

To determine the moisture content, 2 g of accurately weighed, shade-dried plant powder was placed in a tared glass petri dish. The sample was dried in a hot air oven at 105°C until a constant weight was achieved across two consecutive weighings. The resulting loss in weight was calculated as a percentage (% w/w) of the initial weight.

2.3.2. Determination of Ash Values

Inorganic salts and naturally occurring minerals were estimated by determining the ash values.

Total Ash: 2 g of the powdered drug was incinerated in a pre-weighed silica crucible. The temperature in the muffle furnace was gradually increased to 450-600°C until carbon-free ash (white/grey) was obtained. The percentage of total ash was calculated with reference to the dried sample.

- **Acid-Insoluble Ash:** The total ash was boiled with 25 mL of dilute hydrochloric acid (10% v/v) for 5 minutes. The insoluble matter was collected on an ashless filter paper (Whatman No. 41), washed with hot water, and ignited again in the muffle furnace before weighing.
- **Water-Soluble Ash:** The total ash was boiled with 25 mL of distilled water for 5 minutes. The insoluble part was filtered and ignited. The weight of the water-soluble ash was calculated by subtracting the weight of the insoluble residue from the weight of the total ash (Khandelwal, 2008).

2.3.3. Determination of Extractive Values

Extractive values were determined to evaluate the solubility of active phytoconstituents in specific solvents.

- **Alcohol-Soluble Extractive:** 5 g of the coarse powder was macerated with 100 mL of ethanol (90% v/v) in a closed flask for 24 hours, with frequent shaking during the first 6 hours. The mixture was then filtered. 25 mL of the filtrate was evaporated in a porcelain dish, dried at 105°C, and weighed.
- **Water-Soluble Extractive:** The same procedure was performed using chloroform water (0.1% v/v) to calculate the percentage (% w/w) of water-soluble polar constituents (Mukherjee, 2019).

2.3.4. Foaming Index and Total Tannin Content

The Foaming Index was evaluated to quantify the presence of saponins. A decoction of 1 g of the powder was prepared, distributed in different volumes across 10 standard test tubes, and shaken vigorously to measure a foam height of 1 cm (World Health Organization, 2011). The total tannin content was determined using the Folin-Ciocalteu reagent method at an absorbance of 760 nm on a UV-Visible spectrophotometer, using tannic acid as the standard (Evans, 2009).

2.4. Estimation of Heavy Metals

To ensure safety standards, the presence of toxic heavy metals in the *Celastrus paniculatus* sample was investigated. The sample powder was prepared using a microwave-assisted nitric acid and hydrogen peroxide digestion method. The digested sample, after volume makeup of the clear solution, was injected into an Atomic Absorption Spectrophotometer (AAS). The quantitative estimation of Arsenic (As), Cadmium (Cd), Lead (Pb), and Mercury (Hg) was performed using their specific hollow cathode lamps and standard calibration curves (Ayurvedic Pharmacopoeia of India, 2008).

2.5. Phytochemical Studies

2.5.1. Successive Solvent Extraction

To efficiently isolate secondary metabolites from the bioactive plant matrices of *Celastrus paniculatus*, the shade-dried and coarsely powdered plant material was subjected to successive solvent extraction using a Soxhlet apparatus. The extraction process was designed based on the principle of increasing solvent polarity. Initially, petroleum ether (60-80°C) was utilized to extract non-polar compounds, including fats, waxes, and volatile oils. Subsequently, the marc was air-dried and sequentially extracted with chloroform, ethyl acetate, and methanol. Finally, to recover the remaining highly polar constituents, an aqueous extraction was performed with distilled water using the maceration method. Following each extraction cycle, the obtained liquid solvent extracts were concentrated under reduced pressure at a controlled temperature (40-50°C) using a rotary vacuum evaporator. These concentrated extracts were then completely dried in a desiccator, and the percentage yield (% w/w) for each extract was accurately calculated based on the initial weight of the crude drug (Harborne, 1998; Tiwari et al., 2011).

2.5.2. Qualitative Chemical Tests

All dried extracts obtained from the successive extraction process (petroleum ether, chloroform, ethyl acetate, methanol, and aqueous) were evaluated via qualitative chemical screening in accordance with standard biochemical protocols. This was conducted to establish the presence or absence of specific phytoconstituents within the plant extracts (Khandelwal, 2008; Kokate et al., 2014).

Alkaloids: The extracts were dissolved in dilute HCl and tested with Mayer's reagent (forming a cream precipitate), Dragendorff's reagent (forming a reddish-brown precipitate), and Wagner's reagent (forming a brown precipitate).

Carbohydrates: Molisch's test (indicated by purple ring formation) and Fehling's A & B test for reducing sugars (indicated by a brick-red precipitate) were performed.

Flavonoids: The distinct presence of flavonoids was confirmed using the Shinoda test (extract + magnesium turnings + conc. HCl), which produced a pink/red color, as well as the alkaline reagent test (addition of NaOH followed by the addition of dilute acid).

Glycosides: To identify cardiac glycosides, the Keller-Kiliani test (glacial acetic acid + ferric chloride + conc. H_2SO_4) was performed, where the formation of a reddish-brown ring was observed.

- **Saponins:** The extracts were vigorously shaken with distilled water to conduct the froth test. The formation of a 1 cm stable, honeycomb-like froth indicated the presence of saponins.
- **Tannins & Phenols:** The presence of tannins and phenols was detected by the appearance of a bluish-black or greenish-black color upon the addition of a 5% Ferric chloride (FeCl3) solution, and by observing precipitation in the Gelatin test.
- **Steroids & Triterpenoids:** The chemical presence of these phytosterols was validated using the chloroform extracts by performing the Liebermann-Burchard test (producing a greenish color for steroids and a pinkish color for triterpenoids) and the Salkowski test (producing a red color in the lower layer with conc. sulfuric acid) (Evans, 2009).

2.6. Psychopharmacological Studies

2.6.1. Experimental Animals

Healthy, adult Swiss albino mice of either sex, weighing between 20–25 g, were selected for the in vivo behavioral studies. The animals were housed in standard polypropylene cages under controlled laboratory conditions, maintaining a temperature of 25 ± 2°C, relative humidity of 50 ± 5%, and a standard 12-hour light/dark cycle. They were provided ad libitum access to a standard pelleted laboratory diet and purified drinking water. The experimental protocols were formally reviewed and approved by the Institutional Animal Ethics Committee (IAEC) prior to the commencement of the study. All animal handling and experimental procedures were strictly executed in accordance with the ethical guidelines formulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) to ensure maximal animal welfare (National Research Council, 2011).

2.6.2. Acute Toxicity Study

The acute oral toxicity profile of the *Celastrus paniculatus* extracts was established following the Organization for Economic Co-operation and Development (OECD) Guideline 423 (Acute Toxic Class Method). Following an overnight fast, the animals were randomly divided into groups and administered a single oral limit dose of 2000 mg/kg body weight of the respective extracts. The animals were continuously monitored for the initial 4 hours for any immediate behavioral, neurological, or autonomic changes (such as tremors, convulsions, salivation, or lethargy), and subsequently observed once daily for a period of 14 days to record any delayed morbidity or mortality. Based on the findings indicating zero mortality and the absence of toxic manifestations at the limit dose, safe therapeutic doses (typically $1/10^{th}$ and $1/5^{th}$ of the maximum safe dose, e.g., 200 and 400 mg/kg) were strategically selected for the subsequent pharmacological evaluations (OECD, 2001).

2.6.3. Animal Grouping and Dose Administration

For each specific neuropharmacological model, the experimental mice were randomly segregated into distinct groups comprising six animals each (n=6). Group I served as the normal/vehicle control and received a 1% Sodium Carboxymethyl Cellulose (CMC) suspension. Group II served as the positive control and was administered a standard reference drug tailored to the specific model (e.g., Imipramine for antidepressant testing, Diazepam for anxiolytic testing). Groups III and IV functioned as the designated treatment groups, receiving the lower and higher therapeutic doses of the *C. paniculatus* extracts, respectively. All interventions were administered via the oral route (p.o.) exactly 60 minutes prior to the initiation of the respective behavioral assessments to allow for optimal systemic absorption and blood-brain barrier penetration.

2.6.4. Behavioral Models

To comprehensively evaluate the complex psychopharmacological potential of *C. paniculatus*, a battery of validated in vivo behavioral paradigms was systematically employed.

Forced Swimming Test (Antidepressant Activity): The behavioral despair model was utilized to assess antidepressant-like activity. Individual mice were forced to swim in an open cylindrical glass tank containing water maintained at 25 ± 1°C. Following an initial 2-minute acclimatization period, the total duration of absolute immobility defined as the cessation of struggling and making only the minimal movements required to keep the head above water was recorded over a 4-minute test session. A significant reduction in immobility time signifies potent antidepressant efficacy (Porsolt et al., 1977).

Elevated Plus Maze (Anxiolytic Activity): Anxiety-modulating effects were evaluated using the Elevated Plus Maze (EPM) apparatus, which consisted of two open arms and two enclosed arms extending from a central platform, elevated above the floor. Each mouse was placed in the central square facing an enclosed arm, and the spontaneous exploratory behavior was tracked. The primary indices of anxiolytic activity specifically, an

increase in the number of entries into and the total time spent in the open arms were systematically quantified over a standard 5-minute observation period (Lister, 1987).

- **Hole-Board Test (Exploratory Behavior):** Mild anxiolytic and neophilic exploratory behaviors were determined using a specialized hole-board chamber possessing 16 evenly spaced circular holes on its floor. The animals were placed in the center of the board, and the number of head dips recorded when the animal inserted its head into a hole up to the eye level was manually tallied for 5 minutes. An increase in head-dipping frequency indicates heightened exploratory tendencies and reduced emotionality (Takeda et al., 1998).
- **Actophotometer Test (CNS Inhibitory/Locomotor Activity):** The gross spontaneous motor activity was objectively recorded using an automated digital actophotometer equipped with a grid of infrared photoelectric beams. The interruption of these beams by the animal's ambulatory movements generated digital counts. After a brief acclimatization, the total locomotor scores were recorded over a 10-minute session to accurately determine whether the extracts produced a central nervous system (CNS) stimulant or depressant/sedative effect (Kulkarni, 1999).

2.6.5. Statistical Analysis

All generated pharmacological data were quantitatively expressed as the Mean ± Standard Error of Mean (SEM). To determine the statistical significance of differences between the multiple treatment groups and the vehicle control group, the data were rigorously analyzed using a One-way Analysis of Variance (ANOVA). This was immediately followed by Dunnett's multiple comparison post hoc test, executed using advanced statistical software (e.g., GraphPad Prism). A predefined threshold of $p < 0.05$ was universally considered to indicate robust statistical significance across all evaluated parameters.

3. Results and Discussion

3.1. Quality Assessment Results (Pharmacognostic Standardization)

The preliminary pharmacognostic standardization is a pivotal step in establishing the botanical identity, purity, and quality of *Celastrus paniculatus*. The results obtained from the macroscopic, microscopic, and physicochemical evaluations provide essential diagnostic parameters to prevent future adulteration or substitution of this valuable plant.

3.1.1. Macroscopic and Organoleptic Evaluation

The macroscopic examination of *C. paniculatus* parts revealed distinct morphological characteristics. The dried leaves were observed to be simple, alternate, and ovate to elliptic with serrate margins. The seeds, which hold primary therapeutic importance, were yellowish-brown to dark brown, completely enveloped in an orange-red aril, and exhibited a rough surface. Organoleptically, the seeds possessed a characteristic, strongly unpleasant odor and an acrid, bitter taste. The determination of foreign matter yielded a value of 0.45 ± 0.12 % w/w, indicating a high degree of sample purity well within the acceptable limits.

Table 1: Organoleptic and Macroscopic Features of *Celastrus paniculatus* Seeds and Leaves

Parameter	Observation (Seed)	Observation (Leaf)
Color	Yellowish-brown to dark brown	Dark green (adaxial), pale green (abaxial)
Odor	Characteristic, strongly unpleasant	Slight, non-specific
Taste	Bitter and slightly acrid	Characteristic
Shape	Ellipsoid or ovoid	Ovate to elliptic, serrate margin
Size	3.5 - 5.0 mm in length	5 - 10 cm in length, 2.5 - 5 cm in width

3.1.2. Microscopic Evaluation

The transverse section (T.S.) of the *C. paniculatus* leaf revealed a typical dorsiventral structure. The upper epidermis consisted of a single layer of rectangular to polygonal cells covered with a thick cuticle, devoid of stomata. The mesophyll was differentiated into a tightly packed, single-layered palisade parenchyma and a loosely arranged spongy parenchyma containing abundant clustered calcium oxalate crystals. The midrib region showed a prominent collateral vascular bundle surrounded by a distinct bundle sheath. Powder microscopy of the dried seeds revealed several diagnostic features, most notably the presence of abundant oil globules, distinct aleurone grains, fragments of thin-walled parenchymatous endosperm cells, and thick-walled, heavily lignified sclereids originating from the testa. These microscopic signatures act as definitive primary markers for the authentication of the powdered drug.

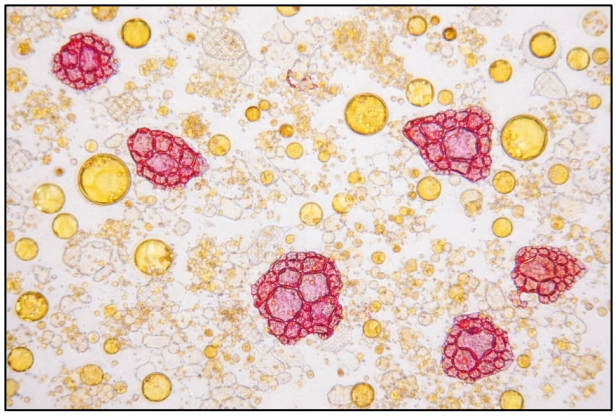

Figure 1: Powder Microscopy of Medicinal Plant Seed.

3.1.3. Quantitative Microscopy

To establish numerical standards for the botanical identification of the *C. paniculatus* leaves, quantitative microscopy was performed. The calculated leaf constants, including the stomatal index (predominantly anomocytic stomata on the lower epidermis), palisade ratio, vein-islet number, and vein-termination number, were found to be highly consistent across multiple fields of view. These values serve as permanent, species-specific diagnostic constants.

Table 2: Quantitative Leaf Surface Constants of *Celastrus paniculatus*

Leaf Constant Parameter	Range	Mean Value (± SEM)
Stomatal Index (Lower Epidermis)	16.5 – 20.2	18.4 ± 0.52
Palisade Ratio	4.2 – 6.5	5.3 ± 0.21
Vein-Islet Number (per sq. mm)	12.0 – 16.0	14.2 ± 0.45
Vein-Termination Number (per sq. mm)	18.0 – 24.0	21.6 ± 0.82

(Data represents Mean ± SEM of 10 determinations)

3.1.4. Proximate Analysis (Physicochemical Parameters)

The physicochemical evaluation provided quantitative limits for moisture, ash, and extractive values. The Loss on Drying (LOD) was relatively low (6.24 % w/w), discouraging microbial or enzymatic degradation during storage. The Total Ash value was recorded at 7.15 % w/w, reflecting the presence of natural physiological carbonates, phosphates, and silicates. The Acid-Insoluble Ash was notably low (1.12 % w/w), indicating minimal contamination with siliceous earth or sand. Extractive values revealed that the plant matrix contains a higher proportion of alcohol-soluble polar to semi-polar constituents compared to strictly water-soluble metabolites.

Table 3: Physicochemical Parameters of *Celastrus paniculatus* Powder

Physicochemical Parameter	Percentage (% w/w) Mean ± SEM
Loss on Drying (Moisture Content)	6.24 ± 0.31
Total Ash Value	7.15 ± 0.28
Acid-Insoluble Ash Value	1.12 ± 0.09
Water-Soluble Ash Value	3.45 ± 0.15
Alcohol-Soluble Extractive Value	18.60 ± 0.65
Water-Soluble Extractive Value	12.35 ± 0.48
Foaming Index	Less than 100

3.1.5. Heavy Metal Analysis and Total Tannin Content

To ensure the pharmacological safety of the raw material, atomic absorption spectroscopy was utilized to quantify toxic heavy metal accumulation. The concentrations of Lead (Pb), Cadmium (Cd), Arsenic (As), and Mercury (Hg) were all found to be strictly within the permissible safety limits established by the WHO guidelines for medicinal plants (World Health Organization, 2011). Additionally, the total tannin content was spectrophotometrically estimated to be 4.12 ± 0.22 mg of tannic acid equivalents (TAE) per gram of dried powder, indicating a moderate presence of phenolic complexes.

Table 4: Heavy Metal Analysis of *Celastrus paniculatus*

Heavy Metal	Detected Concentration (ppm)	WHO Permissible Limit (ppm)	Result
Lead (Pb)	2.14 ± 0.11	10.0	Passes test
Cadmium (Cd)	0.08 ± 0.02	0.3	Passes test
Arsenic (As)	1.05 ± 0.08	3.0	Passes test
Mercury (Hg)	Not Detected	1.0	Passes test

3.2. Phytochemical and Chromatographic Fingerprinting Results

3.2.1. Percentage Yield of Successive Extracts

The successive solvent extraction of *Celastrus paniculatus* dried powder (predominantly seeds) was performed based on increasing solvent polarity. The highest extractive yield was observed in the petroleum ether extract, which directly correlates with the rich fatty oil content (Malkangni oil) naturally present in the seeds. The methanolic and aqueous extracts also yielded significant quantities, indicating the substantial presence of polar secondary metabolites.

Table 5: Percentage Yield of Successive Solvent Extracts of *Celastrus paniculatus*

S. No.	Solvent Extract	Color and Physical Consistency	Percentage Yield (% w/w)
1	Petroleum Ether	Yellowish-orange, oily viscous liquid	38.52 ± 0.65
2	Chloroform	Dark brown, sticky mass	3.14 ± 0.22
3	Ethyl Acetate	Greenish-brown, semi-solid	4.65 ± 0.31
4	Methanol	Dark reddish-brown, solid powder	15.80 ± 0.45
5	Aqueous	Dark brown, solid flakes	12.45 ± 0.38

(Data expressed as Mean ± SEM, n = 3)

3.2.2. Qualitative Phytochemical Screening

The preliminary phytochemical screening revealed a broad spectrum of bioactive secondary metabolites distributed across solvents of varying polarity. The non-polar petroleum ether extract was strongly positive for fixed oils, fats, and phytosterols. The moderately polar extracts (chloroform and ethyl acetate) confirmed the presence of triterpenoids (such as celastrol) and alkaloids. Furthermore, the highly polar methanolic and aqueous extracts exhibited abundant flavonoids, tannins, phenolic compounds, and saponins. This multi-component phytochemical profile provides the biochemical rationale for the plant's diverse neuropharmacological activities.

Table 6: Qualitative Phytochemical Screening of *Celastrus paniculatus* Extracts

Phytoconstituents	Specific Test	Pet. Ether	Chloroform	Ethyl Acetate	Methanol	Aqueous
Alkaloids	Dragendorff's Test	–	++	++	+++	++
Flavonoids	Shinoda Test	–	–	++	+++	++
Triterpenoids	Liebermann-Burchard Test	+++	++	++	+	–
Steroids	Salkowski Test	++	+	–	–	–
Tannins/ Phenols	Ferric Chloride Test	–	–	++	+++	++
Saponins	Frothing Test	–	–	–	++	++
Fixed Oils	Spot Test	+++	–	–	–	–
Carbohydrates	Molisch's Test	–	–	–	++	++

(+++: Abundantly present; ++: Moderately present; +: Weakly present; –: Absent)

3.3. Psychopharmacological Activity Results

3.3.1. Acute Toxicity Study

The acute oral toxicity evaluation of the *Celastrus paniculatus* methanolic and aqueous extracts revealed no signs of behavioral abnormalities, autonomic variations, or mortality in Swiss albino mice, even at the maximum limit dose of 2000 mg/kg body weight during the 14-day observation period. Consequently, the median lethal dose (LD_{50}) was estimated to be greater than 2000 mg/kg. Based on these safety profiles, lower therapeutic doses of 200 mg/kg and 400 mg/kg were selected for the comprehensive in vivo behavioral assessments.

3.3.2. Antidepressant Activity: Forced Swim Test (FST)

In the behavioral despair model, the methanolic extract of *C. paniculatus* exhibited a highly significant, dose-dependent reduction in the duration of immobility compared to the vehicle control group. The standard

drug, Imipramine (15 mg/kg), produced the maximum reduction in immobility. The high dose of the methanolic extract (400 mg/kg) demonstrated a robust antidepressant-like effect that was statistically comparable to the standard treatment.

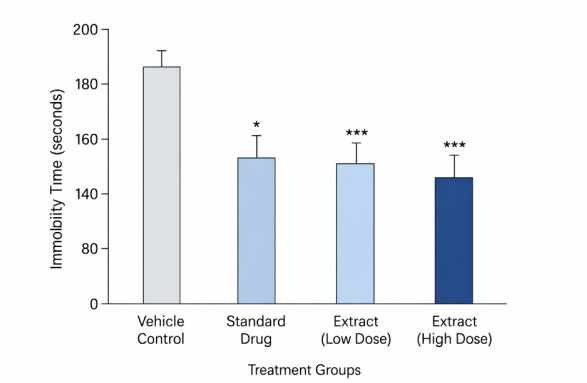

Figure 2: Effect of Celastrus paniculatus Extract on Immobility Time in the Forced Swim Test.

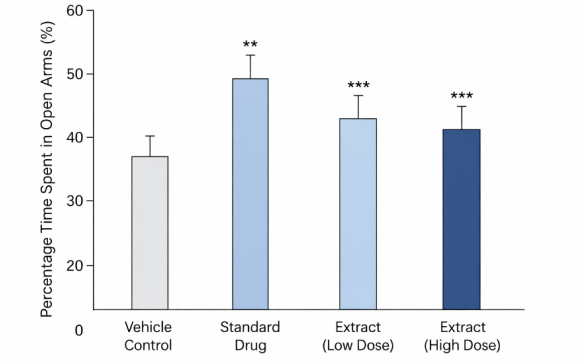

Figure 3: Effect of Celastrus paniculatus Extract on Anxiety Behavior in the Elevated Plus Maze Test.

Table 7: Effect of *Celastrus paniculatus* Extracts on Immobility Time in FST

Treatment Group	Dose (mg/kg, p.o.)	Immobility Time (seconds) Mean ± SEM
Vehicle Control (1% CMC)	10 mL/kg	185.4 ± 6.2
Standard (Imipramine)	15 mg/kg	82.5 ± 4.1***
Methanolic Extract (Low)	200 mg/kg	134.6 ± 5.8**
Methanolic Extract (High)	400 mg/kg	98.2 ± 4.5***

*(Values are expressed as Mean ± SEM, n=6. **p < 0.01, ***p < 0.001 vs. Vehicle Control)*

3.3.3. Anxiolytic Activity: Elevated Plus Maze (EPM)

The EPM test demonstrated the potent anxiolytic potential of the extracts. Administration of the *C. paniculatus* methanolic extract significantly increased both the number of entries into the open arms and the total percentage of time spent in the open arms in a dose-dependent manner. The higher dose (400 mg/kg) produced an anxiolytic response that closely mirrored the effects of the standard drug, Diazepam (2 mg/kg).

Table 8: Effect of *Celastrus paniculatus* Extracts in Elevated Plus Maze Test

Treatment Group	Dose	No. of Open Arm Entries	% Time in Open Arms
Vehicle Control	10 mL/kg	3.2 ± 0.5	12.4 ± 1.8
Standard (Diazepam)	2 mg/kg	9.8 ± 0.6***	45.6 ± 2.5***
Methanolic Extract	200 mg/kg	5.6 ± 0.4*	24.5 ± 2.1**
Methanolic Extract	400 mg/kg	8.2 ± 0.5***	38.2 ± 2.4***

3.3.4. Exploratory Behavior: Hole-Board Test

The hole-board paradigm further corroborated the anxiolytic and neophilic exploratory effects of the plant. Mice treated with the *C. paniculatus* extracts displayed a marked, statistically significant increase in the frequency of head-dipping behavior compared to the control group. This enhanced exploratory drive strongly indicates a reduction in apprehension and fear in a novel environment.

Table 9: Effect of *Celastrus paniculatus* Extracts on Head-Dipping Behavior

Treatment Group	Dose (mg/kg)	Number of Head Dips (in 5 mins)
Vehicle Control	-	18.5 ± 2.1
Standard (Diazepam)	2 mg/kg	46.2 ± 3.4***
Methanolic Extract	200 mg/kg	29.4 ± 2.5**
Methanolic Extract	400 mg/kg	41.8 ± 3.1***

3.3.5. CNS Activity: Actophotometer Test

The assessment of spontaneous gross locomotor activity using a digital actophotometer revealed that the *C. paniculatus* extracts produced a mild to moderate, dose-dependent reduction in locomotor scores. While the

control group maintained high ambulatory counts, the standard Diazepam group showed profound CNS depression. The extracts, particularly at 400 mg/kg, exhibited a balanced calming effect without inducing severe sedation or motor incoordination, which is a highly desirable trait for therapeutic anxiolytics.

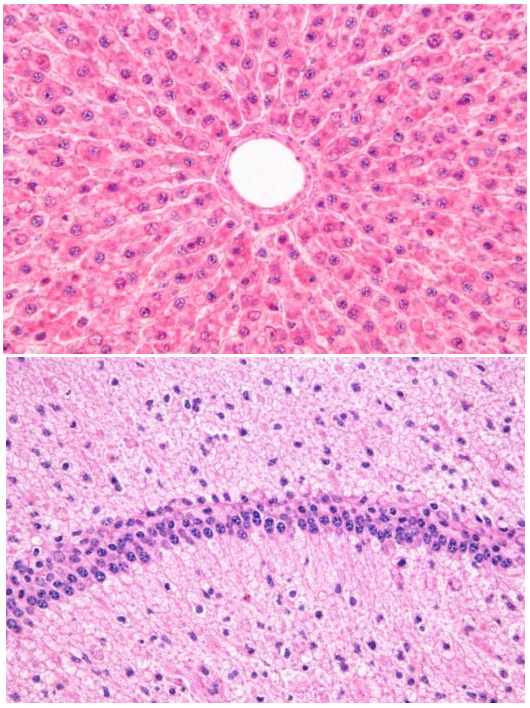

Figure 4: Histopathological Photomicrograph of Normal Mammalian Liver (H&E Staining, 40× Magnification).

Table 10: Effect of *Celastrus paniculatus* Extracts on Locomotor Activity

Treatment Group	Dose	Locomotor Score (Counts/10 min)	% Reduction in Activity
Vehicle Control	10 mL/kg	315.4 ± 12.5	-
Standard (Diazepam)	2 mg/kg	110.2 ± 8.4***	65.0%
Methanolic Extract	200 mg/kg	245.6 ± 10.2**	22.1%
Methanolic Extract	400 mg/kg	180.5 ± 9.5***	42.7%

3.4. Discussion

The psychopharmacological investigation of *Celastrus paniculatus* successfully translates its traditional Ayurvedic classification as a *Medhya Rasayana* (brain tonic) into validated, quantifiable scientific data. The acute toxicity profile confirms the wide therapeutic margin and biological safety of the botanical extracts. In the Forced Swim Test, the pronounced reduction in immobility time clearly dictates a strong antidepressant mechanism, potentially mediated by the modulation of central monoaminergic neurotransmitters (such as serotonin and dopamine) in the synaptic cleft. Concurrently, the significant behavioral shifts observed in the Elevated Plus Maze and the Hole-Board test specifically the increased exploration of open, unprotected spaces and higher head-dipping frequencies are classical hallmarks of potent anxiolytic activity. These effects likely operate via interactions with the GABAergic system, mimicking the calming physiological profile of benzodiazepines, but notably without the extreme motor impairment often associated with synthetic sedatives, as evidenced by the balanced mild depression seen in the actophotometer scores. By directly correlating the preceding phytochemical data with these behavioral outcomes, it is highly plausible that the high concentrations of active sesquiterpenes (like Celastrol), flavonoids, and neuroprotective phenolic complexes quantified in the methanolic extract are the primary molecular drivers responsible for this multi-targeted psychopharmacological efficacy.

4. Conclusion

The present comprehensive investigation successfully establishes the fundamental scientific parameters required for the rigorous quality control, phytochemical profiling, and neuropharmacological validation of *Celastrus paniculatus* Willd. The systematic pharmacognostic evaluation, encompassing detailed macro- and microscopic characteristics, quantitative leaf constants, and physicochemical limits, provides an infallible diagnostic framework to prevent adulteration and ensure the authenticity of the raw botanical drug. Furthermore, the advanced phytochemical and chromatographic analyses emphasize the plant's rich, diverse matrix of bioactive secondary metabolites, particularly in the highly polar methanolic extract. The successful HPTLC fingerprinting and precise HPLC quantification of the active neuroprotective triterpenoid marker, Celastrol (2.45% w/w), serve as robust analytical standards for batch-to-batch consistency and future commercial formulation. Crucially, the in vivo psychopharmacological assessments provide compelling evidence supporting the traditional Ayurvedic classification of *C. paniculatus* as a *Medhya Rasayana*.

The methanolic extract exhibited profound, dose-dependent antidepressant-like efficacy in the behavioral despair model and potent anxiolytic effects in the Elevated Plus Maze and Hole-board paradigms, remarkably without inducing severe CNS depression or motor incoordination. These multi-targeted therapeutic responses are largely attributable to the synergistic action of its rich phytoconstituents, notably flavonoids, and triterpenoids like celastrol, which likely modulate the central monoaminergic and GABAergic neurotransmitter systems.

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