

Free Radical Scavenging Efficiency of *Celastrus paniculatus*: Comparative Analysis of Leaf-Derived Extracts and Seed Oil

Ritika Mehra and *Neetu Pandey

School of Applied Chemistry and Basic Sciences, Sardar Bhagwan Singh University, Dehradun, Uttarakhand, India

*Email: neetu_bhett@yahoo.co.in

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Abstract–Free radicals induce oxidative stress in the human body, which is directly linked to many chronic diseases such as cancer, heart disease, and neurological problems. Nowadays, plants are a great source of natural antioxidants because they include many bioactive phytochemicals like flavonoids, alkaloids and phenolic compounds that help neutralize free radicals. In recent years, plant-derived antioxidants have attracted significant attention due to their potential therapeutic applications and low risk of adverse effects. Among these, one is *Celastrus paniculatus*, sometimes referred to as the intellect tree or Malkangani. It is adaptable and can be used to treat a range of illnesses due to its diverse phytoconstituents. The present study aimed to evaluate and compare the antioxidant potential of leaf extracts obtained through successive solvent extraction and the seed oil of *Celastrus paniculatus*. Leaf samples were subjected to successive solvent extraction using solvents of increasing polarity, while seed oil was obtained through standard extraction methods. The antioxidant activity of the extracts and seed oil was assessed using the in vitro DPPH free radical scavenging

assay, with ascorbic acid used as the standard reference, which is also used as the standard antioxidant, which has a IC_{50} value ranging approximately 10-35ppm.

The results revealed that the seed oil exhibited strong radical scavenging activity with an IC_{50} value of approximately 34 ppm, indicating the highest antioxidant potential among the tested samples. Among the leaf extracts, the methanolic extract showed good antioxidant activity with an IC_{50} value of approximately 42 ppm, followed by the other extracts, which demonstrate moderate antioxidant potential.

These findings suggest that the seed oil of *Celastrus paniculatus* possesses greater antioxidant properties and is comparable to the standard antioxidant, and represents a promising natural source of antioxidants for potential therapeutic applications.

Keywords: Oxidative stress, Antioxidants, *Celastrus paniculatus*, IC_{50} .

Introduction

Free radicals, or reactive oxygen species (ROS), formed within the body, such as superoxide anion, hydroxyl radical, and hydrogen peroxide, are highly reactive

and potentially damaging chemical species^[1]. A natural mechanism used by all aerobic organism, including humans to deal with the process of oxidation used a series of antioxidant defence system which is naturally present in our body, such as a preventive antioxidant system that reduces the rate of free radical formation and chain breaking antioxidant that neutralize and stabilize the free radical these can be enzymatic or non-enzymatic^[2] but if somehow they are not able to manage or control the free radical generation and they exceeds the normal range than that might lead to potential risk in the body such as cellular damage, DNA disorder, cancer, aging etc. Antioxidants are compounds that inhibit or delay the oxidation of molecules, particularly lipids, thereby preventing the formation and propagation of free radicals. Anti-oxidants can be broadly classified based on their origin and method of preparation into natural and synthetic types^[3]. Synthetic antioxidants have been widely used in the food and pharmaceutical industries for many years due to their effectiveness and stability. However, growing concerns regarding their potential toxicity and adverse health effects have led to increased scrutiny and a gradual shift in preference toward natural alternatives. Natural antioxidants not only perform the essential function of preventing oxidation but also exhibit additional health-promoting properties, including anti-inflammatory, anti-microbial and cardioprotective effects. Extensive research has demonstrated that plant materials are rich sources of natural antioxidant phytochemicals, especially flavonoids and other polyphenolic

compounds. These bioactive constituents play a crucial role in neutralizing free radicals and interrupting chain reactions, thereby reducing oxidative stress and lowering the risk of disease development.

Among such plants, *Celastrus paniculatus* is one of the most common, which is also known as Jyotishmati or Malkangani or Black oil plant. It is a traditional climbing shrub belonging to the family Celastraceae, widely used for several medicinal activities in ayurvedic and Unani systems^[4,5]. In Ayurveda, Siddha and Unani, parts of the *Celastrus paniculatus* are used to prevent various diseases like brain-related disorders, arthritis, paralysis, asthma, cancer^[6], stimulant nerve tonic, rejuvenant, sedative, tranquillizer, and diuretic. It is also used in the treatment of rheumatism, gout, leprosy, leucoderma, paralysis and asthma^[7].

In India, *Celastrus Paniculatus* is an evergreen endangered medicinally important plant also known as the elixir of life due to its cognitive boosting properties, which makes it interesting and also important as it will be a natural source of antioxidant where, causing no harmful effects to us. Due to this, the plant is selected for the analysis of its antioxidant potential and from those part which is most widely used.

Material and Methods

Preparation of the sample- Leaves of *Celastrus paniculatus* were collected from the local area of Tehri Garhwal, and seeds were collected from the local market of Dehradun, Uttarakhand. The seeds and leaves were dried in the sun and shade, and crushed respectively. Both of the materials were subjected to

Soxhlet extraction using different solvents, and excess solvent was removed and extracts were prepared.

Extraction of Seeds of *C. paniculatus*-

The seeds were dried in the shade and used for analytical work. About ~50 gm of shade dried seeds were crushed with mortal pestle. The material was then filled in the thimble for the Soxhlet apparatus and exhaustively extracted with petroleum ether for about 9 hours. The solvent was distilled off at low temperature and concentrated on a water bath to get a thick syrup after extraction with Petroleum ether

Chemicals and instruments used - For the extraction process and evaluation, different solvents were used, with the following names: DPPH for evaluation of antioxidant potential; Methanol, Chloroform, Pet ether, Aluminium Chloride, NaOH, Sodium Nitrite for TFC and chemicals for Phytochemical screening. For the evaluation, a UV-Visible Spectrometer, Soxhlet and a distillation apparatus were used.

Qualitative Analysis of the Phytochemicals

- i. Test for Tannins-** This was done by boiling extract samples (separately) in water in a test tube and then filtering. A brownish-green or blue-black colouration was observed after adding a few drops of 0.1% ferric chloride.
- ii. Test for Saponins-** To detect the presence of saponins, the Foam Test was performed. The *Celastrus Paniculatus* extract was diluted with distilled water and heated to a boil. Once cooled, the solution was shaken vigorously. The formation of a

persistent foam was taken as a positive indicator for the presence of saponins.

iii. Test for Flavonoids- **a.** This was determined through heating of the extract of the *Celastrus* leaves sample (separately) with ethyl acetate over a steam water bath for a few min.

b. To dilute the ammonia solution, filtrate from the filtered mixture was added and shaken. The appearance of yellow colouration indicates the presence of flavonoids.

c. Ferric chloride test- To the test solution, add a few drops of ferric chloride solution, intense green colour was formed to show the presence of flavonoids.

iv. Test for Steroids- This was carried out by the addition of a little amount of acetic anhydride to each of the crude extracts with further addition of H_2SO_4 . The presence of steroids was indicated by a change of colour from violet to blue or green.

v. Test for Terpenoids- This was carried out by Salkowski's test. To the extract was added chloroform, followed by the careful further addition of concentrated H_2SO_4 . Appearance of red colour at the interface is an indication of a positive result for the presence of terpenoids.

vi. Test for Alkaloids- The *Celastrus* leaves extract was taken, to it was added 1% HCl, mixed, warmed and later filtered. Mayer's and Dragendorff's reagents were added to the filtrate, and the absence or presence was determined based on appearance of the turbidity or precipitate development.

Preparation of DPPH Solution - The free radical scavenging activity was determined using the in-vitro DPPH

assay. 0.7mg of DPPH was dissolved in 100 mL of Methanol for the free radical, this will be the working solution. The solution was mixed thoroughly until complete dissolution and stored in the dark at low temperature for 2 hours for further use.

Preparation of Extract Solutions- Stock solution of different extracts (Leaf-Methanol, chloroform, pet ether and Seed) of 1 mg/mL was prepared by dissolving the required quantity of each extract in the required volume of methanol. From the sample stock solutions 50, 100, 150, 200 and 250 μg / mL sol. of each extract were prepared.

Evaluation of Antioxidant Potential- Take the sample solutions of different concentrations. 2 mL DPPH solution and 2 mL of the extract solution were mixed and incubated at room temperature in the dark for 2 hours. A Blank solution was also prepared, only using 2 mL of DPPH solution. Finally, the absorbance of the solutions was measured by a UV-visible

spectrophotometer at 517 nm. Ascorbic acid was used as the standard. IC_{50} values, which is 50% inhibition of the free radicals of the extracts, were calculated from the graph as concentration v/s % inhibition. Radical scavenging activity was expressed as a percentage of inhibition. IC_{50} value is the concentration of the sample required to scavenge 50% of DPPH free radical. IC_{50} of the extracts indicates the corresponding concentration at which the radical scavenging potential is 50%. The IC_{50} of the extract and standards were determined graphically.

The percentage of inhibition was calculated by using the formula:

$$\text{I\%} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where,

A_{Control} = absorbance of the control

A_{sample} = absorbance of the sample sol.

I% = percentage of inhibition.

The radical scavenging activities of the extracts are expressed in terms of their IC_{50} values.

Results

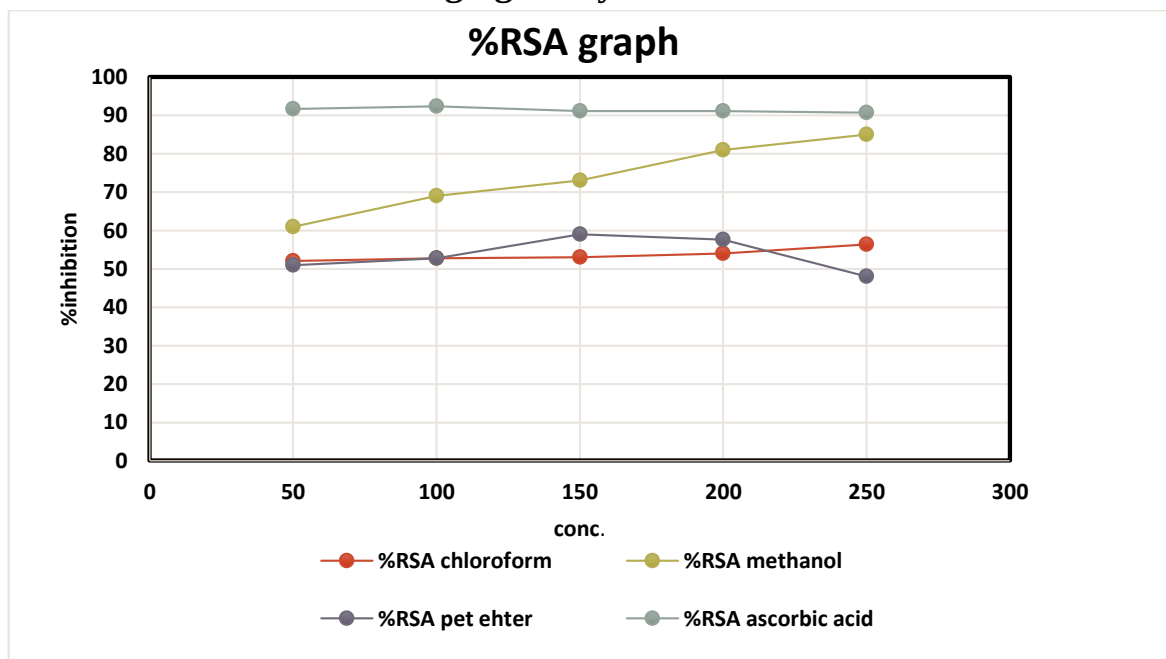
Table-1 Qualitative Analysis of Leaves of *Celastrus p.*

Phytoconstituents	Methanol	Chloroform	Pet ether
Tannins	+	-	-
Saponins	+	-	-
Steroids	+	+	+
Flavonoids	+	-	-
Terpenoids	+	+	+
Alkaloids	+	Trace	-

Table-2 Qualitative Analysis of the Seed of *Celastrus p.*

Phytoconstituents	Absence(-)/Presence(+)
Tannins	-
Saponins	-
Steroids	+
Flavonoids	-
Terpenoids	+
Alkaloids	-

DPPH Free Radical Scavenging Assay



Graph- 1 showing % Inhibition of the different solvent extracts of *Celastrus paniculatus*

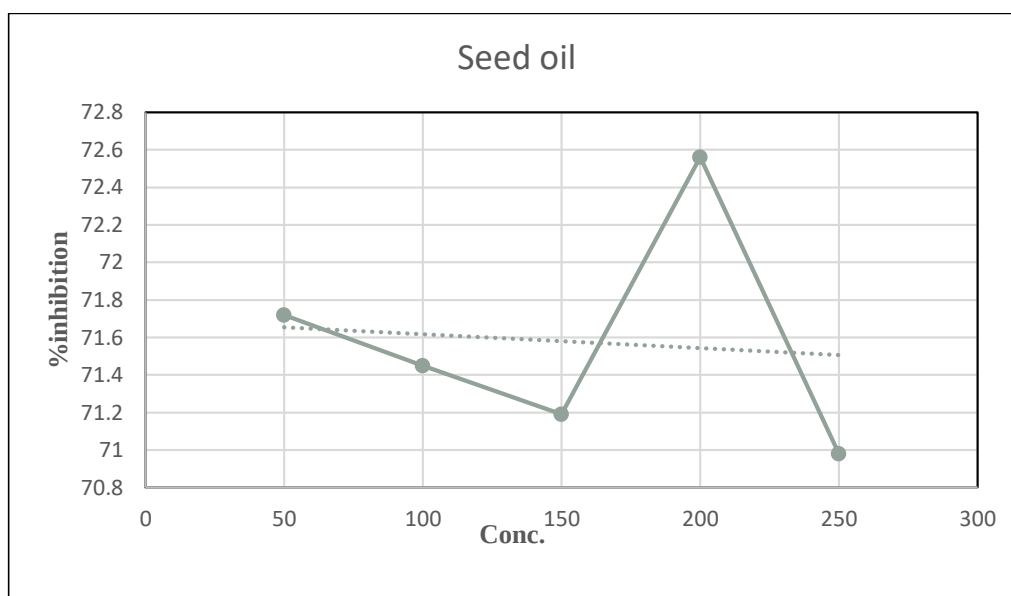
Radical Scavenging Activity (%RSA)

of Leaf Extracts and Seed Oil- The antioxidant potential of *Celastrus paniculatus* leaf extracts was evaluated using different solvent systems: methanol, chloroform, and petroleum ether. Ascorbic acid served as the standard, maintaining a consistently high inhibition rate of approximately 90-92% across all tested concentrations (50 to 250 ppm). (Graph- 1)

Among the leaf extracts, the methanol extract exhibited the highest antioxidant activity, showing a clear concentration-dependent increase from 60.8% at 50ppm to 84.9% at 250ppm. In contrast, the chloroform and Pet Ether extract showed significantly lower activity, around 52-56% and 47-58.9%, respectively. In comparison to the methanol extract and standard, it showed lower inhibition.

Graph-2 showed the inhibition percentage of seed oil demonstrated a high baseline of antioxidant activity, with % inhibition ranging between 71% and 72.6%. Unlike the methanol leaf extract, the seed oil did not show a strong positive correlation with concentration. The highest inhibition (72.57%) was recorded at 200ppm, followed by a slight decrease at higher concentrations.

While there is a higher percentage of inhibition in the methanol leaf extract, overall, looking at the IC₅₀ value, which is the value where the substance will show 50% of inhibition in free radical scavenging. Methanol showed a value of 42ppm, Chloroform, and Pet ether have an IC₅₀ value, while the seed oil showed a value of 34ppm, and the standard has an IC₅₀ value ranging from 10-35ppm.



Graph-2 Showing %Inhibition of Seed Oil of *Celastrus paniculatu*

Discussion

This data demonstrates that in % inhibition, Methanol exhibits the highest value reaching up to 84%, from which we can say that the primary antioxidant metabolites of the leaves are polar in nature. The methanol extract contains alkaloids, flavonoids, and phenolic compounds, which are known to have antioxidant properties by donating a hydrogen atom to stabilise free radicals, whereas Chloroform and Petroleum ether have very limited antioxidant capacity due to their nonpolar constituents. The seed oil showed a good antioxidant property. At its lowest concentration, it also showed higher inhibition of free radical also it has a higher value of IC_{50} value also which shows the efficacy of the seed oil has higher antioxidant. The seed oil serves as a more consistent and potent source of radical scavengers.

Conclusion

The present comparative study between leaves and seeds of *Celastrus paniculatus* highlights clear differences in both phytochemical content and anti-oxidant

potential. The methanolic leaf extract exhibited higher total flavonoid content (84.09 mg QE/g) compared to the seed sample (59.01 mg QE/g), indicating that leaves are a richer source of flavonoid compounds when extracted with methanol.

In terms of antioxidant activity, a distinct variation was observed between the two plant parts. The seed oil showed the highest antioxidant potential among all tested samples, demonstrating strong free radical scavenging activity with a low IC_{50} value (~35 ppm). This suggests that seeds contain potent lipid-soluble antioxidant compounds. On the other hand, the methanolic leaf extract also showed significant antioxidant activity with a relatively low IC_{50} value (~41 ppm), indicating a strong contribution from polar phytochemicals such as flavonoids and phenolics. Therefore, overall antioxidant potential was higher in seeds (oil) than in leaves."

Overall, the study concludes that both leaves and seeds of *Celastrus paniculatus* are valuable sources of anti-oxidant compounds but differ in their phytochemical profiles and efficiency. Seeds are more potent in terms of overall antioxidant activity, while leaves are richer in flavonoid content. This comparative evaluation suggests that different plant parts may be utilized for specific therapeutic or nutraceutical applications depending on the desired bioactive compounds.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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