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ORIGINAL ARTICLE **OPEN ACCESS**

Pharmacognostical, Phytochemical and Antioxidant Study of *Celastrus paniculatus* Willd. from North-Western Himalayas in Himachal Pradesh

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ABSTRACT
North-Western Himalayas zone is an abundant source of valuable medicinal and aromatic plants. Celastrus paniculatus is a traditional Ayurvedic medicinal plant and also known as Jyotishmati in Sanskrit from this region. The present research work aimed to determine the pharmacognostical evaluation and reducing power antioxidant assay of Celastrus paniculatus whole plant except roots from the north-western Himalayan range of Himachal Pradesh. Successive solvent extraction was executed with ethanol, acetone, chloroform and water to carry out an antioxidant assay. The cross-section and powder microscopical study showed the various characters like different trichomes, epidermal arrangements, fibers and other microscopical features of seed, stem and leaf. Physiochemical parameters were within the limits of WHO guidelines. Water-soluble extractive value (21.80% w/w) was greater than other solvents. Preliminary phytochemical screening of successive solvent extracts showed the presence of several secondary metabolites phytosterols, phenols, flavonoids, terpenoids, carbohydrates and proteins. By the evaluation of IC_{50} (Half maximal inhibitory concentration) values of all extract it's clear that the water (IC_{50} 87.03 µg·mL-1) and ethanolic (IC_{50} 95.02 µg·mL-1) extracts have the best antioxidant activity (IC_{50} of CPW < CPE <CPA< CPC). Low IC_{50} values indicate high antioxidant activity. Different standard antioxidants like Vitamin-C and quercetin were used to compare the outcome of test samples. This study showed the pharmacognostical standards for identification and codify of Celastrus paniculatus and confirmed that the water and ethanol extracts of Celastrus paniculatus from the north-western Himalayas is a good source of antioxidant drug.
KEYWORDS: *Celastrus paniculatus, Ayurvedic medicinal plant, Antioxidant activity, Jyotishmati, Pharmacognostical evaluation*

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INTRODUCTION

Family, Celastraceae is widespread in the tropical and semi-tropical regions all over the globe and is covered approximately 88 genera, cover shrubs, liana plants and trees [1,2]. *Celastrus paniculatus* Willd. (CP) is one of the members of the Celastraceae family and genus Celastrus, traditionally known as Jyotishmati or malkangani and is a highly endangered plant [2,3].

Celastrus paniculatus is a medicinally significant large, woody, climbing shrub of about 10 to 18 m in height. The ripped fruits of the CP are yellow with reddish-brown seeds shielded with a scarlet aril. CP seeds reported better in taste and hot in potency. CP is available roughly all over the mountainous region of India up to an elevation of 2000m. However, CP is also grown wildly in Sri Lanka, Nepal, China, Malaysia, Australia, Thailand, Indonesia and in the Pacific islands. [5,6].

All parts of CP may be utilized concerning its nutritional and remedial values. In Ayurveda, it is used as an influential brain tonic, laxative, digestive, diuretic, emmenagogue, expectorant and diaphoretic [7]. It is also observed that seeds of CP are very useful for skin disease, wounds healing, scabies, leukoderma, cardiac debility, cough, asthma, amenorrhea, epilepsy, gouts, paralysis, inflammatory, and dysmenorrhea and utilized by tribals in various part of India. Fruits of CP and its oil helpful to cure amenorrhea, diarrhea, skin disease, and dysentery, whereas roots of CP are worked against scorpion bite [8-10]. Recent past in-vivo studies showed that different solvent extracts of the seeds of CP have shown antioxidant and anti-inflammatory potent on mice. Due to the high antioxidant capacity, seed extracts

have shown the high cognitive enhancing activity on rats [10, 11].
As per our knowledge by literature survey, most of the scrutinize on the seed of CP from various segment of India and this is the first detailed pharmacognostical and preliminary anti-oxidant study of whole plant of *Celastrus paniculatus*, which is collected from the north-western Himalayas in Himachal Pradesh to see the pharmacognostical characters and to see the anti-oxidant study of *Celastrus paniculatus* of north-western Himalayan region.

MATERIAL AND METHODS

The whole plant (except roots) of CP were collected from north-western Himalayas (latitude: 31.991240° N, longitude: 76.789917° E) at Joginder Nagar, H.P. India, the elevation of 1,220 meters and confirms authenticity by Prof. D. N. Sharma, (Former Director) Research Institute of Indian System of Medicine, Joginder Nagar, Mandi, H.P. and the voucher specimen (ACP/RSY/2018/15) has been preserved for further utilization. The leaves stem and fruits parts were washed cleanse and dried under shade for 4 weeks for further use.

Macroscopy

The following macroscopical (morphological) constitution of the fresh stem, fruits, seed and leaves were noted: surface, shape, color, odor, texture, taste and size.[12].

Microscopy

The freehand fine cross-sections of the fresh leaves, seed and stem were entertained with various staining agents and monitor the regular and specific microscopical constitution. Furthermore, a small amount of the leaves, seed and stem was cleared, powdered, mounted and monitor for various powder microscopical characteristics [13].

2.3 Physicochemical investigations

The dried plant material examined for foreign organic matter, moisture content, Sand &silica. The dried CP drug was made coerce powder and utilized for the calculation of varied extractive and ash values [14]

Preparation of extract by successive solvent extraction

Preparation of extract by consecutive solvent extraction 250 g of the powdered whole plant was subjected to consecutive solvent extraction using soxhlet apparatus such as Pet. ether, Chloroform, Acetone and Water for 6 hrs and named as CPE, CPC, CPA and CPW respectively. Every time before extraction with consecutive solvent the powdered plant drug was dried overnight. All the extracts (CPE, CPC, CPA, CPW) were subjected to removal of the excess solvent by rotatory vacuum evaporator at 45-50 °C. They were then air-dried. The coloration and consistency of the extracts were noted. The extracts prepared were used for the phytochemical and experimental antioxidant study.

Qualitative Phytochemical Analysis:

All four extracts (CPE, CPC, CPA, CPW) were introduced to preliminary qualitative phytochemical screening. Different phytochemical was performed to scan the presence of various phytoconstituents [15, 16]

Antioxidant activity by reducing power assay

The reducing strength of CPE, CPC, CPA and CPW was ascertained according to the method described previously [19]. The different concentrations of CPE, CPC, CPA and CPW (50–250 μg·mL-1) in distilled water (1 ml) was mixed with phosphate buffer (pH 6.6, 2.5 ml, 0.2 mol·L-1,) and potassium ferricyanide [K3Fe(CN)6] (2.5 ml, 1%) and the mixtures incubated at 50°C for 20 min. A 2.5 ml quantity of 10% trichloroacetic acid was incorporated into the mixtures, which were then centrifuged for 10 min at 3 000 r·min-1. The top layer of the solutions (2.5 ml) was combined with distilled H_2O (2.5 ml) and 0.1% FeCl3 (0.5 ml), and the absorbance was assessed at 700 nm in a UV spectrophotometer (UV-1601 Shimadzu, Japan). Strong absorbance of the reaction mixture suggests greater reducing power [17].

Statistical analysis

All the quantitative test procedures were repeated three times. Results data were demonstrated as mean ± SEM (n=3). Statistical interpretation was calculated with a one-way interpretation of all sample variance (ANOVA) accompanied by post hoc "Dunnett's Multiple Comparison Test" using Graph Pad Prism software package. $P < 0.05$ were scrutinize statistically important.

RESULT

Macroscopy

Fruits *Celastrus paniculatus* (CP) are simple septifragal valvular dehiscence capsular fruits, which are yellow to deep orange, stalked, globose, about 8 to 13mm in diameter. Each fruit contains 3-6 seeds in three locules joined to the central axile column.
Seeds are enclosed in orange-red aril. Without the aril, seed size ranges 5-6.5 mm in length and 2.5- 3.5 in

breadth. Seeds are cinnamon-brown in color, oval, bilateral, glabrous with horny tests, hilum apical, acute/pointed, thick, scaly circular cells, cellular reticulations. Seeds are roughly 3 sides, convex on one side and the other 2 are converging towards each other, but two side seeds, with one convex and one more or less flat side.

Stem are climbing shrub, unarmed or unbranched with pendulous fruit-bearing branches. Stems are upto 2.2 cm in diameter and 8-10 m long with rough, pale yellow-brown color and encircled densely with macro, elongated lenticels. Fresh leaves upper surface are sea green and lower surface are light greenish, odorless with a faintly acrid taste.

The leaves were simple, orbicular, elliptic in shape, alternate in arrangement, the apex is mucronate, acuminate or cuspidate and the base is rounded, the margin is finely serrate, the venation is pinnate. The leaves were glabrous and glaucous, average leaf size is 9-10 cm length and 6-7 cm breadth.

Microscopy

The microscopical study of seed shows wavy outer epidermis trailed by 2-3 layers of pigmented parenchyma cells forming the thick cell wall of seed. Below the cell wall, thick cells(arranged compactly) with tanniferous inclusion were present. This region is followed by a palisade layer made up of elongated cells with broad intercellular space in between each cell followed by endosperm region containing oil globules (Figure 1A-C).

The cross-section of leaf midrib and part of lamina shows in figure 2A-C. The midrib region(biconvex) shows polygonal upper and lower epidermis having cuticles and stomata. The epidermis had 2types of trichomes namely unicellular and multicellular (uniseriate with rounded head) and lower epidermal cells were smaller compare with upper epidermal cells with arch-shaped (anticlinal) walls. Below the upper and lower epidermis 2-3 layers of collenchyma were presently followed by distinguishable parenchyma. The vascular bundles were c-shaped confiding pink (lignified) color circular xylem and non-lignified phloem fibers surrounded by a 4-5paranchyma fiber zone. The rhomboid calcium oxalate was present in ground parenchymatous tissue.

The microscopical feature of the stem was shown in figure 3A-C. The cross-section was almost circular in outline. The outer layer of stem called epidermis was single-cell layered with lenticellate, unicellular and multicellular hair. The epidermis was followed by hypodermal 4-5 layer collenchymatous cells without intercellular space. Endodermis followed by hard bast shown2-3 layered pericyclic sclerenchyma. Starch grains and oil ducts were seen in cortical cells. Medullary rays were rounded, angular and thick-walled. The vascular bundle consists of primary, secondary xylem internally and metaxylem, protoxylem center peripherally and phloem externally. The pith contains a well-defined parenchyma cell and forms a central cavity.

Powder Microscopy

Powder characters of seeds were included thicken the part of seed coat containing compact and elongated sclereids with thick lignified secondary walls and narrow lumen. Yellow parenchyma cells were present followed by endosperm with oil globules (Figure 1D-G). Powder leaf of CP was inspected microscopically and observed micro features showed epidermal cells and lignified, thickened, spiral xylem vessels. Non-glandular, unicellular, multicellular, conical covering trichomes were present. Lignified sclerenchymatous fibers from vascular bundles and prism-shaped calcium oxalate crystals (Figure 2D-H). Microscopic of stem powder showed lignified parenchyma cells, medullary rays with attached lignified fibers. Epidermal, cortical cells and stone cells were also present. Spiral and pitted xylem vessels, unicellular and multicellular hairs were observed(Figure 3D-G).

Physicochemical investigations

Dried powdered plant drug was introduced to physicochemical analysis to determine different ash values, extractive values, foreign organic matter, moisture content, Sand and silica and results were tabulated in table 1.

Preparation of Extracts

Successive solvent extracts were prepared and named as CPE (ethanol), CPA (acetone), CPC (chloroform) and CPW (water). The percentage yield, color and consistency of extracts were shown in table 2.

Qualitative phytochemical analysis

Extract CPE, CPA, CPC and CPW were examined for the presence of various phytochemicals and the result was tabulated in table 3.

Antioxidant activity by reducing power assay

The result of reducing the power method of CP extracts compared to quercetin and Vitamin C are shown in figure 4. In the reductive power measurement, the Fe3+ to Fe2+ transformation by every extract samples (CPE, CPC, CPA, CPW) were investigated using a method explained previously [18]. The IC50 of the every extract CPW, CPE, CPC, CPA, quercetin, and Vitamin C were calculated to be 87.03, 95.02, 163.62, 142.71, 67.75 and 83.99 respectively. Water extract revealed higher and significantly reducing

capacity than other extracts but the reductive capability was lesser as compare with quercetin or vitamin C. Reducing ability towards free radicals by the extracts and standard chemicals followed the trend: Vitamin C > quercetin > CPE > CPC > CPW > CPA.

Table 1: Physicochemical investigations of *Celastrus paniculatus* whole plant (except roots).

Parameter	% w/w
Ash values	
Total ash	5.01±0.29
Acid insoluble ash	0.98±0.15
Water-soluble ash	1.56±0.32
Sulphated ash	4.23±0.21
Extractive values	
Alcohol soluble	11.36±0.51
Water-soluble	21.80±0.34
Hexane soluble	1.06±0.12
Ether soluble	2.19±0.42
Extraneous material	
Moisture content	>2%
Foreign matter	0.5%
Sand &silica	Not visible

Table 2: Percentage yield and characteristics of extracts of *Celastruspaniculatus* whole plant (except roots).

Extract	% Yield	Colour	Consistency
Aqueous (CPW)	18.20%	Blackish brown	Sticky
Ethanol (CPE)	12.56%	Light brown	Crystalline
Acetone (CPA)	10.89%	Dark Brown	Sticky
Chloroform (CPC)	2.12%	Cream	Crystalline

Table 3: Phytochemical test of ethanol, chloroform, acetone and water extract of *Celastrus paniculatus* whole plant (except roots).

Phytochemical test	Aqueous extract	Ethanol Extract	Chloroform Extract	Acetone extract
Alkaloids				
Carbohydrates	+(reducing sugar)	+(reducing sugar)	+(reducing sugar)	+(reducing sugar)
Phytosterols	+	+	+	-
Glycosides	-	-	-	-
Flavonoids	+	+	-	+
Saponins	-	-	-	-
Phenols	+	+	-	-
Tannins	-	-	-	-
Proteins	+	+	-	+
Terpenoids	+	+	+	-

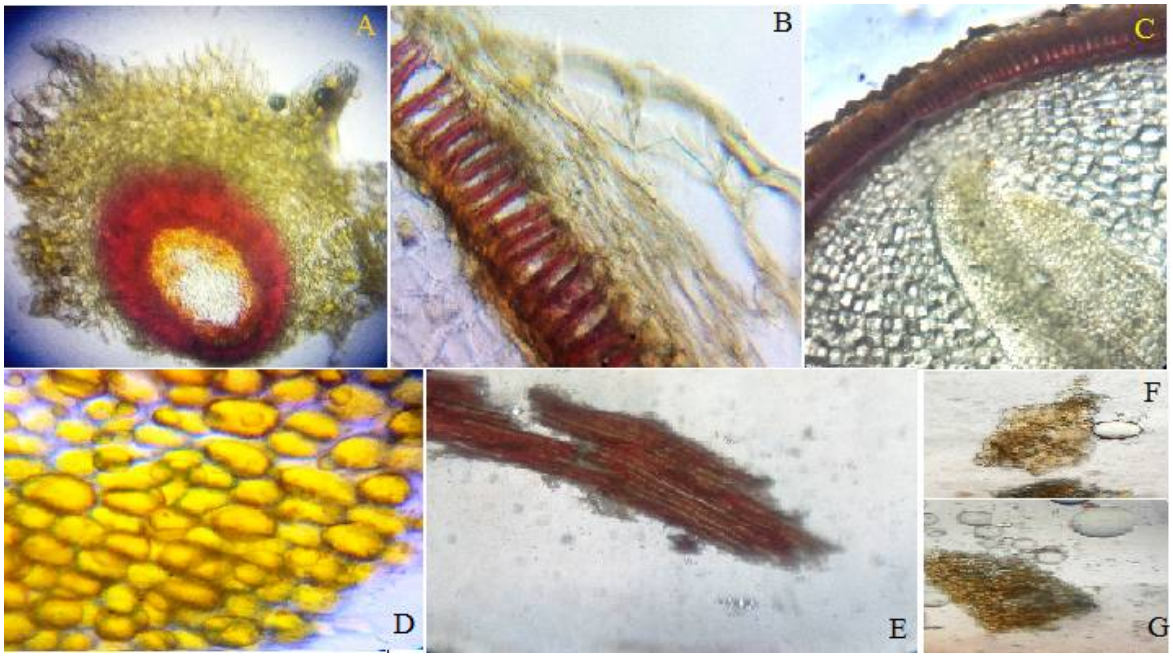

Figure 1:(A) Cross-section of seed of C. paniculatus (B)Wavy epidermis, palisade layer (C) Well developed Endosperm, oil globules (D) Yellow parenchyma cells (E)Sclereids (F,G) Endosperm with oil globules.

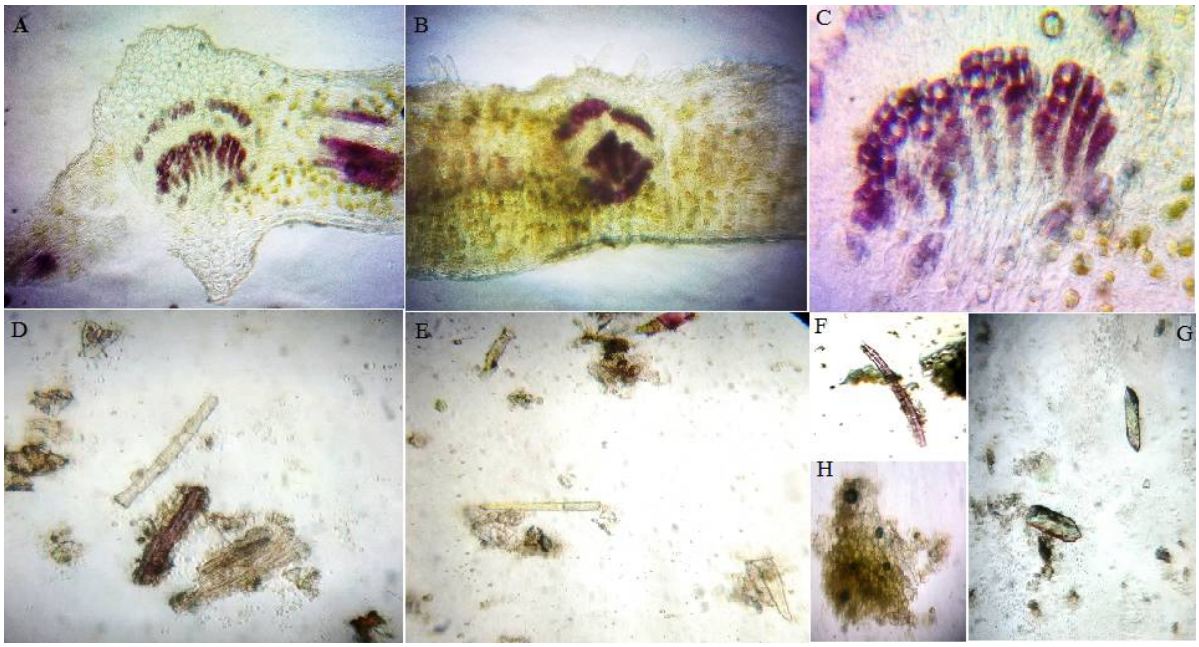

Figure 2:(A) Cross-section of leaf midrib of C. paniculatus (B) Part of lamina (C) Vascular bundles (D)Non-glandular multicellular trichomes (E) Unicellular trichomes (F) Spiral xylem vessels (G)Calcium oxalate crystals (H) Epidermal cells.

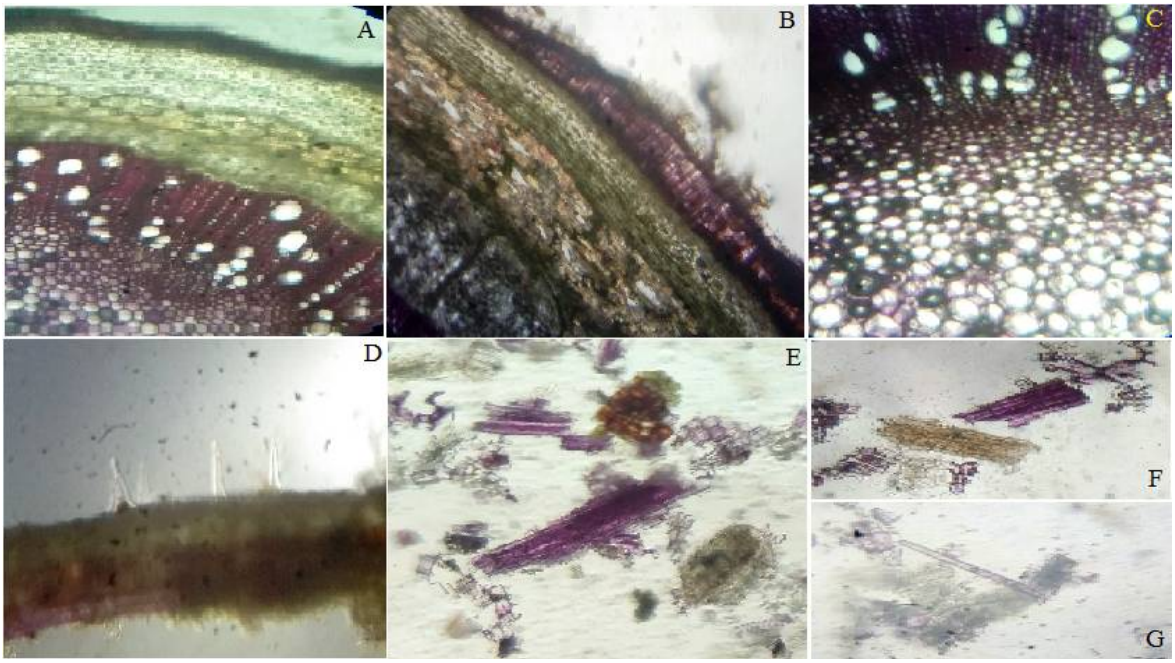

Figure 3:(A) Cross-section of stem of C. paniculatus (B) Epidermis layer with lenticels (C) Vascular bundle, pith(D)Conical covering hairs(E)Lignified parenchyma and cork cell (F)Pitted xylem vessels (G) Multicellular hair.

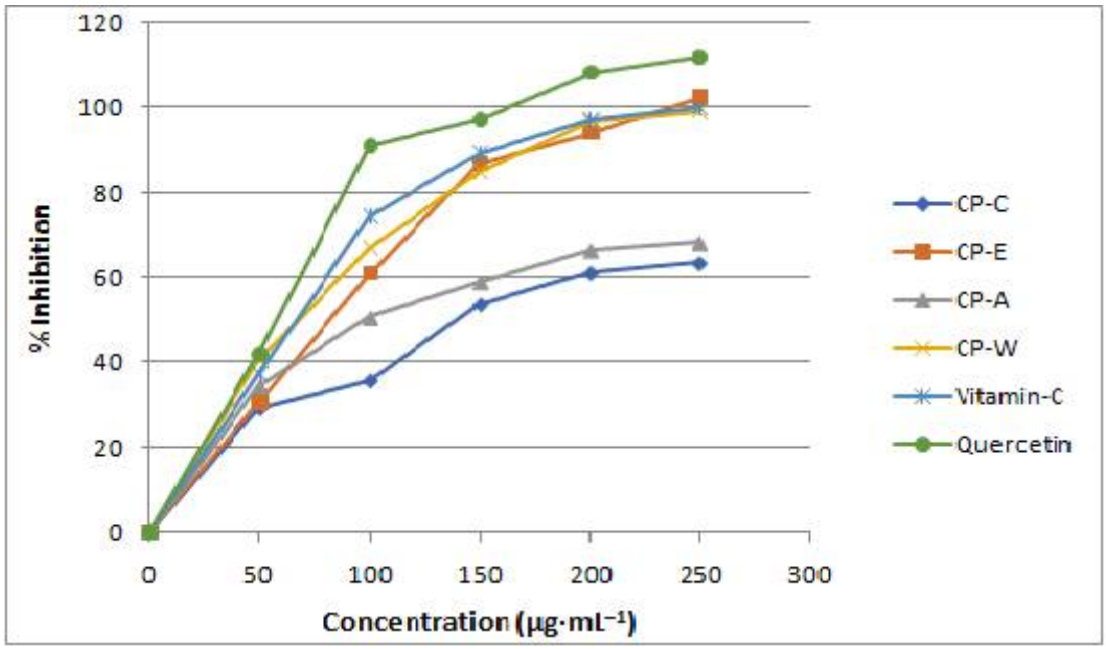

Figure 4: Reducing strength of extracts CPE, CPC, CPA and CPW of *Celastrus paniculatus* whole plant (except roots).Results are Mean ± SEM of three parallel readings.

DISCUSSION

There are many advanced instrumental hyphenated scientific techniques are came up but still, the macro-microscopical and physicochemical study are of great significance, reliable, quick and easy to carryout and inexpensive methods. As per the world health organization (WHO) guidelines, the morphological and microscopical (transverse section and powder drug) evaluation of the plant drug is the primary move towards proving the identity and purity before any other kind of tests are carried out. [18]

Furthermore for the natural drug practitioner or natural researchers and manufacturers identification is most important and macroscopic and microscopic features play great significance for this purpose [19].

In the present work, the morphological and microscopic inspection of *Celastrus paniculatus* was performed from the north-western Himalayan region. The morphological features might help distinguish

Celastrus paniculatus of other geographical regions of the Indian continent and its adulterants. The microscopic study also allows more deep examinations of identification features such as epidermis, parenchymatous cell of a leaf, seed and stems of *Celastrus paniculatus* from this region.

The physiochemical constant has a very significant role to determine the quality and purity of medicinal plant drugs. The foreign organic matters that were present in a very small amount in the stem part may be due to climbing on other plants for support [15]. The moisture content was around >2% % may be due to the presence of a rich amount of oil in seeds. The moisture part should be minimum so that the drug will not decompose soon and protected from microbial attacks. Ash value is also one of the standard parameters to identify the quality of plant drugs because the inorganic impurities in large amounts can harm the patient in long term treatment [20]. Ash value of north-western Himalayan *Celastrus paniculatus* isa very minute amount which is good as per quality and safety standards. The water-soluble extractive value was significantly high as compared with other solvents. Extractive value has great significance to detect inferior or adulterated plant drugs.

The phytochemical investigation manifested the presence of different secondary metabolites in different successive solvent extracts which are known for various therapeutic effects in the human body. The water and ethanol extracts were found a maximum number of secondary metabolites like phytosterols, phenols, flavonoids, terpenoids, carbohydrates and proteins.

The result of reducing power antioxidant assay of *Celastrus paniculatus* expressed that water and ethanol extract manifested the significant antioxidant activity as compared to chloroform and acetone extract. As water and ethanol, extract contain numerous secondary metabolites so the antioxidant activity showed may be due to any specific secondary metabolites as phenols and flavonoids compounds are known for their antioxidant activities or due to synergistic effect of various phytoconstituents.

CONCLUSION

The present study gives the very potential data regarding pharmacognostical evaluation of *Celastrus paniculatus* obtained from the north-western Himalayan range of Himachal Pradesh. Which is necessary for the identification, quality control and standardization of *Celastrus paniculatus* of this region. Phytochemical evaluation pointed out that water and ethanol extract contains a maximum number of different secondary metabolites and significant antioxidant activity. The remarkable antioxidant activity further lighting the path for the use of north-western Himalayan *Celastrus paniculatus* for various neurological disorders majorly in neurosarcoidosis which has no line of treatment till now.

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