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**ANTIOXIDANT ACTIVITY GAUGING OF *CELASTRUS PANICULATUS*
WILLD. FROM NORTH-WESTERN HIMALAYAN REGION OF
HIMACHAL PRADESH**

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ABSTRACT

Celastrus paniculatus Willd. (Cp) from North-Western Himalayas (Family: Celastraceae) is a traditional established Ayurvedic herb. The usage of Cp in medicine dates back to ancient times and also appears in Ayurveda. An investigation is being conducted to find out whether whole plant of Cp (apart from roots) from north-western Himalayas (Himachal Pradesh) can be used for treating neurosarcoidosis in the future. Successive solvent extraction was performed with acetone, chloroform, ethanol and water. Further, *in vitro* antioxidant assessment has been performed which includes Hydroxyl radical (HO·) scavenging, ABTS radical decolorization assay, H₂O₂ scavenging activity, DPPH radical reducing activity, superoxide radical (O₂·⁻) scavenging activity, metal chelating activity, NO· scavenging activity, hydroxyl radical (HO·) scavenging and reducing power assay. By the examination of IC₅₀ (Half maximal inhibitory concentration) value from the *in vitro* antioxidant data of each extract it has been observed that the water extract has the remarkable antioxidant potential. The potency of all the extracts in terms of IC₅₀ follows the orders as CpW<CpE<CpC<CpA. Various standards antioxidants were

used to analyze the data of tested extracts, including Vitamin-C, EDTA, ascorbic acid, metabisulfite, and quercetin. In the present study, water extracts of Cp from north-western Himalayas from Himachal Pradesh were found to contain significant antioxidant levels, which are also in agreement with Ayurveda texts and Indian medical systems. A further study of Cp from this area may prove useful in the treatment of neurosarcoidosis based on this analysis.

Keywords: *Celastrus paniculatus*, Antioxidant, Neurosarcoidosis

INTRODUCTION

Tree of life, Oriental Bittersweet, Black Oil Plant, Jyotishmati and malkangani is associated with the genus *Celastrus* and biologically known as *Celastrus paniculatus* (Family- Celastraceae) [1].

Woody, Climbing shrub, unarmed, flower-bearing with yellow fruits distributed from Western Ghats to Himalayas up to 2000m height (**Drug, 2010; Indian council of medicinal research, 2007**).

Apart from its seeds all parts of *Celastrus paniculatus* (Cp) used for various disorders in different part of India traditionally. It's reported that majorly its seeds are use more over other parts and treated diseases like: inflammation, gout, dysmenorrhea, skin disease, wound healing, asthma, amenorrhea, cardiac problems, leukoderma scabies, cough and well used by the village peoples in India traditionally. Fruits and oil of Cp have tremendous medicinal richness and help to overcome epilepsy, diarrhea, dysentery paralysis and amenorrhea, sometime roots are applied for scorpion bite. Cp is part of

many ayurvedic medicines appetizer, digestive, diuretic, emmenagogue, powerful brain tonic, diaphoretic, febrifuge and laxative [2-4]. Cp seed extracts demonstrated strong remarkable upliftment of cognitive activity on rats because of rich antioxidant capability. In various current findings of Cp have reported the noticeable anti-inflammatory and anti-oxidant potential on mice. After reviewing the various data and literature sources, various types of studies has been conducted on seeds of *Celastrus paniculatus* in all over the India but this present experiment is the thorough antioxidant study with eight different methods for complete screening of anti-oxidant potential of Cp obtained from north-western Himalayas of Himachal Pradesh to explore its future use to treating neurosarcoidosis [5-7].

MATERIALS AND METHODS

Plant Collection and priming of the plant extract:

Whole plant of *Celastruspaniculatus* was used except root part. Cp was procured at Mandi District of Himachal Pradesh (north-western Himalayas) in the month of September (latitude: 31.991240° N, longitude: 76.789917°E) and the elevation was around 1300 meters. First the plant herbarium was made for the identification. Cp was identified and authenticated by Prof. Deenanath Sharma from Research Institute of Indian System of Medicine, Joginder Nagar, Mandi, Himachal Pradesh. Plant was first subjected to remove foreign organic matter, sand, silica and shade dried for 45 days, slashed and powdered coarsely by electric grinding machine. Successive solvent technique was used for extraction with 4 different solvents (Ethanol, acetone, chloroform and water). The same powdered drug was extracted for 8 hr with all solvents one by one in glass soxhlet apparatus and after every extraction the marc kept overnight at room temperature (25-26°C). All the extracts which are denoted as CpE (Ethanol extract), CpA (Acetone extract), CpC (Chloroform extract) and CpW (Water extract) were undergone to rotary flask evaporation to remove excess solvent. Extracts (CpE, CpA, CpC, CpW) were stored in amber color bottles at 4°C for future use to

determine antioxidant and total phenolic content by various methods.

Drugs and chemicals

2, 2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 1, 1-diphenyl-2-picryl hydrazyl hydrate (DPPH) were procured by Sigma-Aldrich (USA). Tripyridyltriazine (TPTZ) was procured through Fluka Chemical Company (Switzerland). Quercetin, acetylsalicylic acid and indomethacin were granted as favor samples through Aura Pharmaceuticals, Panchkula, Haryana. Chemicals for Analytical use were procured through SRL Mumbai and E. Merck (India).

Uncovering of total Phenolic Profile of Cp

Each of the extracts (CpE, CpA, CpC, CpW) was investigated for total phenol by employing Folin-Ciocalteu method and quercetin (accustomed standard [8]). A test piece of (1.0 mL) every extract (CpE, CpA, CpC, CpW) solution (10mg/mL Conc.) were taken and incorporated 46 mL distilled water in various volumetric flask for extracts (separate of every extract) and standard. Approximately 1.0mL Folin-Ciocalteu reagent was subsume in every individual flask (test and sample) and stir well properly. About 3.0mL sodium carbonate (2%) was subsume in all test extracts and standard sample after 3 minute

with frequent shaking for 3 h. all test extracts and standard sample were examined in UV spectrophotometer (UV-1601 Shimadzu) at about 760 nm. The uncovering of total phenolic profile of every extract were denoted by $\text{mg}\cdot\text{g}^{-1}$ of test sample. An equation was obtained by drawing the regression line of standard (quercetin) plot and the total phenols of every sample extract (CpE, CpA, CpC, CpW) were computed as gram of quercetin equivalent.

$$Y = 0.002x + 0.107, r^2 = 0.9270$$

(x denoted the concentration and y denoted absorbance)

Antioxidant activity

Validation of 1,1-diphenyl-2-picrylhydrazyl free radical reducing (DPPH) response

The free radical reducing response of each Cp extract (CpE, CpA, CpC, CpW) was validated by DPPH mediated assay [9]. A $0.1\text{mmol}\cdot\text{L}^{-1}$ DPPH solution was formulate in ethanol and 1 mL solution was subsume in each Cp extract (3 mL) in water at varied concentrations ($50\text{--}250\ \mu\text{g}\cdot\text{mL}^{-1}$) and stir well. The varied blends were blended firmly and each blends rested for 30 min at $25\text{--}26^\circ\text{C}$ (room temperature). The test blends were screened at 517nm after resting in a UV spectrophotometer (UV-1601 Shimadzu, Japan). Slighter absorbance of the test blends

denoted higher the free radical reducing response of Cp extracts. The % DPPH scavenging response was recorded for each Cp extract employing the below equation:

$$\text{DPPH reducing response (\%)} = [(A_0 - A_t / A_0) \times 100]$$

(A_0 = control response absorbance and A_t = Cp extracts response absorbance)

Validation of 2, 2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid radical (ABTS) decolorization evaluation

A $7\ \text{mmol}\cdot\text{L}^{-1}$ concentration solution of ABTS was formulated by subsume in water. A stock solution of ABTS blend with $2.45\ \text{mmol}\cdot\text{L}^{-1}$ potassium persulfate (last concentration) to generate ABTS and rest the reactant blend for 12 to 16 h at $25\text{--}26^\circ\text{C}$ (room temperature) in dark area prior to use. For the examination of Cp extracts, phosphate-buffered saline was utilize for dilution of ABTS stock solution ($5\ \text{mmol}\cdot\text{L}^{-1}$ and pH 7.4) to meet an absorbance (at 734 nm) of 0.70. The decreasing concentration of 1.0 mL ABTS was subsume in each Cp extract ($20\ \mu\text{L}$), rest for next 5 m and then absorbance response were noticed [10]. Current study response were calculated as:

$$\% \text{ ABTS-scavenging activity} = [\text{control response absorbance} - \text{sample response absorbance}] / [\text{control response absorbance}] \times 100$$

Reducing power Validation

Reducing potentiality of (CpE, CpA, CpC,

CpW) was examined by the procedure elaborated prior [11]. The varied concentrations of each Cp extract (50–250 $\mu\text{g}\cdot\text{mL}^{-1}$) dissolved in 1 mL water (distilled) was subsume with 2.5 mL, 6.6 pH 0.2 $\text{mol}\cdot\text{L}^{-1}$ phosphate buffer and 2.5 mL (1%) potassium ferricyanide [$\text{K}_3\text{Fe}(\text{CN})_6$] and rest at 50°C in incubation for about 20 m. A 2.5 mL 10% solution of trichloroacetic acid and every Cp extract blend mixed together and centrifuged for 10 minute (3000 rpm). The 2.5 mL supernatant layer of each blend was again subsume with 2.5 mL mL water (distilled) and 0.5 mL ferric chloride (0.1%), and the each Cp extract solution was screened in UV- spectrophotometer at 700 nm (UV-1601 Shimadzu). The percent reducing potentiality were calculated for every Cp extract employing the below equation:

$$\text{Reducing power response (\%)} = [(A_0 - A_t / A_0) \times 100]$$

(A_0 = control response absorbance, and A_t = Cpextracts response absorbance)

Assay of superoxide radical ($\text{O}_2^{\cdot -}$) scavenging potentiality

Present method is based on the potentiality of the Cp extracts (CpE, CpA, CpC, CpW) to degenerate the expansion of blue formazan. Superoxide radicals were generated by riboflavin, light and nitroblue tetrazolium

(NBT) methods [12]. The entire quantity of the reactant blend of each Cp extract was 3 mL and each 3 mL of current reaction blend carried 0.1 mg nitroblue tetrazolium 50 $\text{mmol}\cdot\text{L}^{-1}$, 7.6 pH sodium phosphate buffer, riboflavin (20 μg), Cpextract (1.0 mL) and EDTA (12 $\text{mmol}\cdot\text{L}^{-1}$). The reaction of every extract blend was started by the incorporation of the reaction blend with distinct concentrations of CpE, CpA, CpC, CpW (50–250 $\mu\text{g}\cdot\text{mL}^{-1}$) for 1.5 min and instantly after the incorporation of Cpextracts, the absorbance response was noted down in UV at 590 nm of each Cp extract sample. Identical process was done without Cp extract for blank study. The % of degeneration of superoxide anion expansion was calculated by the below equation:

$$\% \text{ Inhibition} = (A_0 - A_t / A_0) \times 100$$

(A_0 = control response absorbance and A_t = Cpextracts or standard response absorbance)

Assay of nitric oxide ($\text{NO}\cdot$) scavenging potentiality

NO assay is set up by the principle of Greiss's reaction response [13, 14]. Sodium nitroprusside spontaneously fabricate nitric oxide at biological pH in aqueous solution, which fabricates nitrite ions when contact with oxygen. These nitrite ions generation can be calculated by the Greiss reagent. Scavengers create barrier between oxygen

and nitrite ions and stop the development of nitrite-free radical ions. Sodium nitroprusside ($10 \text{ mmol}\cdot\text{L}^{-1}$) in phosphate-buffered saline were subsume with varied concentrations of CpE, CpA, CpC, CpW extracts blend in methanol and rest for about 150m at in incubation at $25\text{-}26^\circ\text{C}$ (room temperature). With the identical protocol control (with no extract) test blend was also made but with the identical volume of methanol. Post incubation of the test blends, all blends were subsume with 0.1% N- (1-naphthyl) ethylenediaminedihydrochloride), 0.5 mL of Greiss reagent (1% sulphanilamide, and 2% H_3PO_4). The absorbance response of all blends was noticed in UV at 546nm to screening the chromophore developed via diazotization of nitrite by sulphanilamide escorted by attribution with naphthylethylenediamine. The ascorbic acid employing as positive control and screened identical as Cp extract samples with Greiss reagent. The calculation of percentage inhibition was calculated by:

$$\% \text{ Inhibition} = (A_0 - A_t / A_0) \times 100$$

(A_0 = control response absorbance and A_t = Cp extracts or standard response absorbance)

Hydrogen peroxide (H_2O_2) scavenging potentiality

The H_2O_2 scavenging potentiality of CpE, CpA, CpC, CpW was screened by the

method utilized prior [15]. A $2 \text{ mmol}\cdot\text{L}^{-1}$ solution of H_2O_2 was formulated using 7.4 pH phosphate buffer. The H_2O_2 intensity was captured spectrophotometrically by UV at 230 nm . CpE, CpA, CpC and CpW ($50\text{-}250 \mu\text{g}\cdot\text{mL}^{-1}$) was subsume in the H_2O_2 solutions (0.6 mL), utilized phosphate buffer as blank solution without H_2O_2 and absorbance taken down 10 min later at 230 nm . The percentage inhibition computed by below equation:

$$\% \text{ Inhibition} = (A_0 - A_t / A_0) \times 100$$

(A_0 = control response absorbance and A_t = Cp extracts or standard response absorbance)

Metal chelating potentiality

The chelating potentiality of ferrous free radical metal ions for CpE, CpA, CpC, CpW were screened by the method employed prior [14, 16]. Varied concentrations of CpE, CpA, CpC, CpW ($50\text{-}250 \mu\text{g}\cdot\text{mL}^{-1}$) were subsume in 0.05 mL FeCl_2 ($2 \text{ mmol}\cdot\text{L}^{-1}$) solution. 0.2 mL , $5 \text{ mmol}\cdot\text{L}^{-1}$ ferrozines mixed to each Cp extract to initiate the reaction. The sample blends were first agitated strongly and then rest at $25\text{-}26^\circ\text{C}$ (room temperature) for 10 m . The absorbance response was majored in UV at 562nm for all reaction blends. The % of Cp extract to pause the ferrozine-ferrous complex genesis in each Cp test blend was derived with the below equation:

$$\% \text{ Inhibition} = (A_0 - A_t / A_0) \times 100$$

(A_0 = control response absorbance and A_t =

extracts or standard response absorbance), EDTA (Ethylenediaminetetraacetic acid) as chelating agent.

Hydroxyl radical scavenging test

The Cp extract test blends were developed by combining 1 mmol·L⁻¹ 2-deoxy-D-ribose, 0.2 mmol·L⁻¹ phenyl hydrazine in 7.4 pH phosphate buffer and Cp extracts (CpE, CpA, CpC, CpW) in varied concentration of 50–250 µg·mL⁻¹. All the test blends were rested in incubation at 37 °C next 4 hr. Trichloroacetic acid (2.8% *W/V*) solution was inserted in test blends to pause the reaction and centrifugation for 10 min to all Cp test blends at 5 000 r·min⁻¹. The supernatant of each test blends was inserted with an aqueous 1% (*W/V*) thiobarbituric acid. Thiobarbituric acid responsive genesis developed were noted at 532 nm by UV-spectrophotometer [17].

Statistical analysis

All examinations were done in triplicate for best possible statistical results. Results statistics were substantiated as mean ± SEM (n=3). Statistical elucidation was quantified with one-way interpretation of all Cp extracts (ANOVA) followed by post hoc "Dunnett's Multiple Comparison Test" using Graph Pad Prism software facility. P < 0.05 were investigated statistically valuable.

RESULT AND DISCUSSION:

Uncovering of total Phenolic Profile of Cp

Presence of HO component in phenols and phenolics compounds made them significantly useful phytoconstituent with free-radical reducing potential. However it is not true all the time that the antioxidant potential is directly a proposal to the number of phenolics and associated compounds [8]. The number of total phenolic portion present in the varied Cp extracts CpE, CpA, CpC and CpW were noted (22.13±2), (1.34±2), (4.56±2) and (39.35±2) mg respectively (demonstrated as quercetin equivalents per gram of extract).

Antioxidant activity

Validation of 1,1-diphenyl-2-picrylhydrazyl free radical reducing (DPPH) response

Excess generation of any types of free-radicals ions is a peril because the body's defense system cannot defend itself from oxidative pressure and fabricate a various chronic state in the constitution of body system [18]. Outcome of present examination revealed Cp as an impressive free radical scavenger, which can protect the varied free radical-induced destruction in the composition of body's physiological configuration. Determining scavenging potential of firm DPPH free radical for scrutiny of antioxidant action in vitro is the phenomenal acceptable and practiced fast

method compared to other methods. Quercetin and vitamin-C (Vc) were employed as standard radical scavengers in this current examination. The scavenging competence of each Cp extract (CpE, CpA, CpC, CpW) on the DPPH free radical ions was comparably low than that of standards as per the outcome. The calculation of IC_{50} assessment of all samples blends of CpE, CpA, CpC, CpW, quercetin, and vitamin C (as standard) were carry out by employing the equation got by the linear regression investigation. The IC_{50} were majored 106.43, 156.8, 151.16, 98.21, 82.33 and 83.21 $\mu\text{g}\cdot\text{mL}^{-1}$, sequentially (Fig.1-A). Outcome Shown, the IC_{50} of $\text{CpW} < \text{CpE} < \text{CpC} < \text{CpA}$, the extract reporting minimum IC_{50} having good free radical inhibitor or scavenger. The present study revealed that water extract (98.21) reported the lowest IC_{50} and considerable anti-oxidant activity out of each Cp extracts (Table 1).

Validation of 2, 2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid radical (ABTS) decolorization evaluation

Every Cp extracts demonstrated concentration-based scavenging of the $\text{ABTS}^{\cdot+}$ radical. The majored IC_{50} data of CpE, CpA, CpC, CpW quercetin, and Vc were 94.87, 180.28, 149.6, 90.31, 78.52 and 89.36 $\mu\text{g}\cdot\text{mL}^{-1}$, sequentially (Figure 1-B).

Water (CpW) extracts reported the remarkable ABTS radical decolorization potential in a concentration-related manner (Table 1).

Reducing power Validation

The outcome of reducing the power method of Cp extracts with respect to quercetin and Vc are demonstrated in Figure 2-C. In the reductive power quantification, the Fe^{3+} to Fe^{2+} transformation by each Cp extract blend (CpE, CpA, CpC, CpW) were examined engaging a procedure explained previously [18]. The IC_{50} of the each Cp extract CpW, CpE, CpC, CpA, quercetin, and Vc were reported as 96.48, 108.59, 141.74, 146.05, 70.12 and 86.92 $\mu\text{g}\cdot\text{mL}^{-1}$ sequentially. Water (CpW) extract demonstrated higher and significantly reducing capability than other Cp extracts but the reductive strength was lower than quercetin or vitamin C (Table 1). Reducing potential towards free radicals by the Cp extracts and standard used followed the sequence: $\text{Vc} > \text{quercetin} > \text{CpW} > \text{CpE} > \text{CpC} > \text{CpA}$.

Assay of superoxide radical ($\text{O}_2^{\cdot-}$) scavenging potentiality

Phenolic compounds enormously flavonoids and catechins are recognized to be well known antioxidants and superoxide ($\text{O}_2^{\cdot-}$) scavengers. The scavenging potential of

phenolic compounds excessively based upon the number of phenols and site of the OH functional groups in any chemical compound [19, 20]. Varied biological processes in the body's physiological operations are damaged by toxic Superoxide anion free radicals. Curtailment in the number of superoxide anion by the antioxidants reported by the depletion in absorbance at 590 nm.

The outcome reported that the gradual enhancement in concentration of varied Cp extracts and standard in reaction blends demonstrated the increment in % inhibition of developed superoxide free radical (**Figure 2-D**). The IC_{50} data of CpE, CpA, CpC, CpW, Vc, and quercetin were reported 115.24, 195.26, 138.11, 105.13, 95.33 and 83.85 $\mu\text{g}\cdot\text{mL}^{-1}$, sequentially (Table 1). CpW and CpE extract demonstrated noticeable antioxidant response as compare with CpA and CpC extracts.

Assay of nitric oxide ($\text{NO}\cdot$) scavenging potentiality

In this present assay, the reduction of NO by Cp extracts (CpE, CpA, CpC, CpW) was scrutinize by observing the reduction of direct time-dependent nitrite formation in the sodium nitroprusside–PBS plan. The outcome demonstrated the concentration dependent reduction in $\text{NO}\cdot$ by all Cp extracts (CpE, CpA, CpC, CpW) and

standard vitamin C (**Figure 3-E**). The research demonstrated that each concentration of Cp extracts (50–250 $\mu\text{g}\cdot\text{mL}^{-1}$) are a positive influence on nitric oxide free radicals but standard Vc shown the biggest hit compared with Cp extracts. The IC_{50} were recorded by employing the equation obtained from linear regression examination by produced data, and recorded 127.36, 190.36, 148.21, 109.6 and 98.4 $\mu\text{g}\cdot\text{mL}^{-1}$ for CpE, CpA, CpC, CpW and vitamin C sequentially (**Table 1**).

Hydrogen peroxide (H_2O_2) scavenging potentiality

Hydrogen peroxide stimulate the genesis of hydroxyl radicals. Eradicate hydroxyl radicals ($\cdot\text{OH}$) is extremely essential to manage system of life as they attack on most of the biological molecules and varied cellular components to lead human tissue destruction followed by cell death [21, 22]. The potential of Cp extracts at varied concentrations (50–250 $\mu\text{g}\cdot\text{mL}^{-1}$) to scavenge H_2O_2 was recorded. CpW and CpE extracts demonstrated noticeable H_2O_2 scavenging potential when the response analogize in contrast with Ascorbic acid (**Figure 3-F**). The recorded IC_{50} values were 98.68, 183.77, 176.92, 96.87 and 93.28 $\mu\text{g}\cdot\text{mL}^{-1}$ for CpE, CpA, CpC, CpW and ascorbic acid sequentially (**Table 1**).

Metal chelating potentiality

Present assay demonstrated that the genesis of ferrous aggregation with ferrozine reagent was constricted by standard (EDTA) as well as Cp extracts also. Current outcome demonstrated clearly that the chelating potential of each Cp extracts and EDTA was demonstrated as % of metal-chelating potential in concentration (50–250 $\mu\text{g}\cdot\text{mL}^{-1}$) dependent manner (**Figure 4-G**). The IC₅₀ were recorded 133.58, 163.66, 134.38, 101.31 and 95.21 $\mu\text{g}\cdot\text{mL}^{-1}$ for each Cp extract (CpE, CpA, CpC, CpW) and EDTA, sequentially (**Table 1**).

Hydroxyl radical scavenging test:

The hydroxyl free radicals and its associated free radicals are the one of most potential species of the ROS that target more or less

each molecule in the physiological arrangement in the humans [23, 24]. Initiation of its action done by the peroxidation of cell membrane lipids and generate malondialdehyde, which is an indication of bio membranedestruction. Hydroxyl radical's genesis carried out in vivo from water by the heavy transferal of irradiation or from H₂O₂ in a metal initialize action. In vitro, CpE, CpA, CpC, CpW were demonstrated concentration (50–250 $\mu\text{g}\cdot\text{mL}^{-1}$) dependent decrease in the hydroxyl free radicals (**Figure 4-H**). The IC₅₀ were recorded 108.16, 176.11, 164.52, 99.25, 94.21 and 90.95 $\mu\text{g}\cdot\text{mL}^{-1}$ for CpE, CpA, CpC, CpW, quercetin, and sodium metabisulfite, sequentially (**Table 1**).

Table 1 Scavenging potential of Cp extracts against following in vitro antioxidant methods (means \pm SEM, n=3)

Drugs (50-250 $\mu\text{g}\cdot\text{mL}^{-1}$)	IC ₅₀ / $\mu\text{g}\cdot\text{mL}^{-1}$							
	DPPH radical	ABTS radical	Reducing power	Superoxide radical	Hydroxyl radical	H ₂ O ₂ scavenging	NO· scavenging	Metal chelating activity
CpA	156.8 \pm 0.16	180.28 \pm 0.56	146.05 \pm 1.06	195.26 \pm 0.87	176.11 \pm 0.35	183.77 \pm 0.56	190.36 \pm 0.20	163.66 \pm 1.01
CpC	151.16 \pm 1.03	149.6 \pm 0.71	141.74 \pm 0.13	138.11 \pm 1.21	164.52 \pm 1.09	176.92 \pm 0.34	148.21 \pm 0.29	134.38 \pm 0.83
CpE	106.43 \pm 0.51	94.87 \pm 0.62	108.59 \pm 0.44	115.24 \pm 0.38	108.16 \pm 0.15	98.68 \pm 0.91	127.36 \pm 0.29	133.58 \pm 0.76
CpW	98.21 \pm 0.45	90.31 \pm 0.14	96.48 \pm 0.68	105.13 \pm 0.51	99.25 \pm 0.05	96.87 \pm 0.16	109.6 \pm 0.18	101.31 \pm 0.36
Vitamin C	83.21 \pm 1.01	89.36 \pm 0.50	86.92 \pm 0.26	95.33 \pm 0.67	94.21 \pm 0.39		98.4 \pm 1.15	
Quercetin	82.33 \pm 0.23	78.52 \pm 1.11	70.12 \pm 0.82	83.85 \pm 0.69				
Metabisulfite					90.95 \pm 0.13			
Ascorbic acid						93.28 \pm 0.37		
EDTA								95.21 \pm 0.77

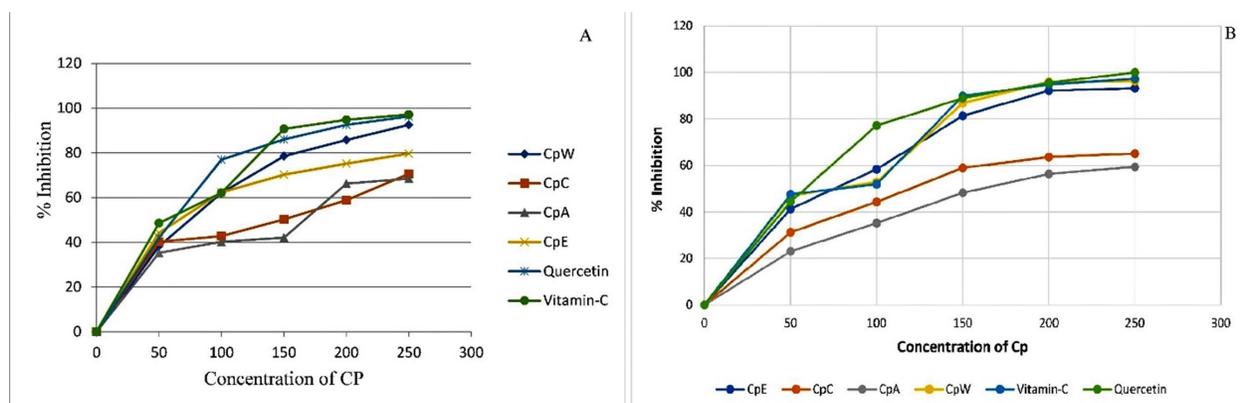


Figure 1: Percentage Inhibition of DPPH (A) and ABTS (B) radicals by different Cp extracts and standard compounds

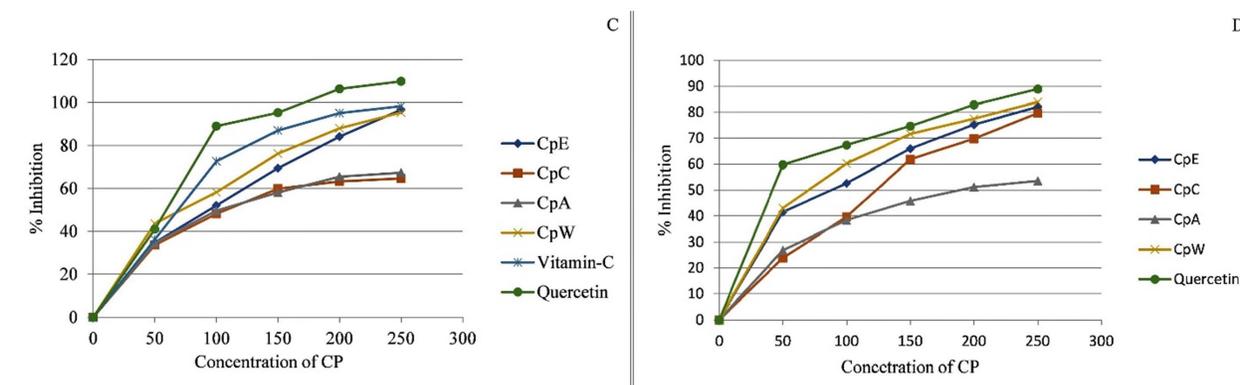


Figure 2: Reducing potential (C) and superoxide radical inhibition (D) activity of Cp extracts and standard compounds

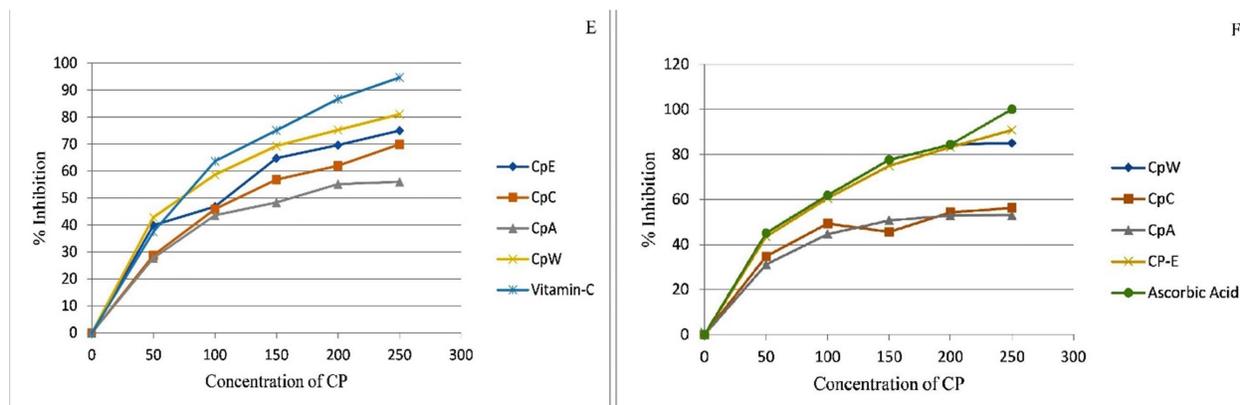


Figure 3: Nitric oxide (E) and H₂O₂ (F) scavenging activity of different extracts of Cp and standard compounds.

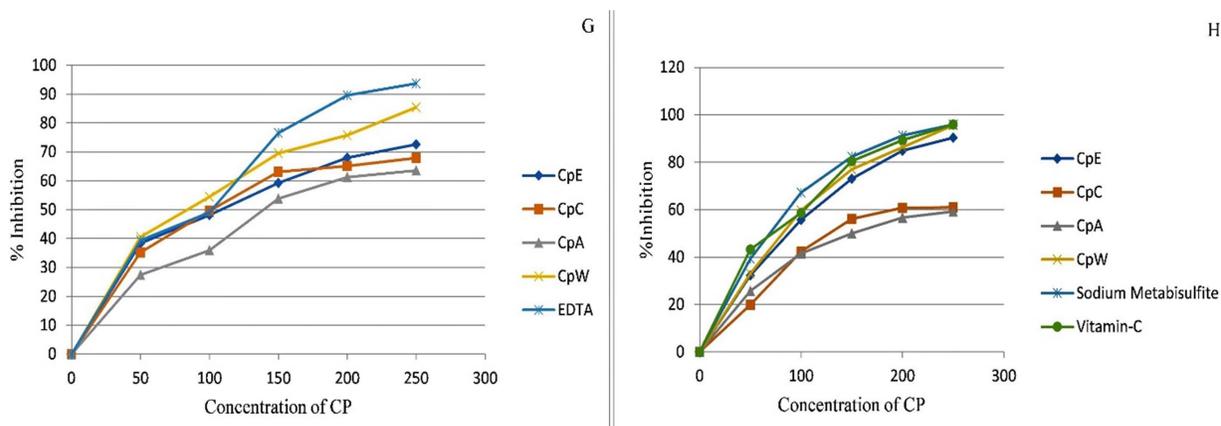


Figure 4: Metal chelating (G) and hydroxyl radical (H) inhibition activity of various Cp extracts and standards

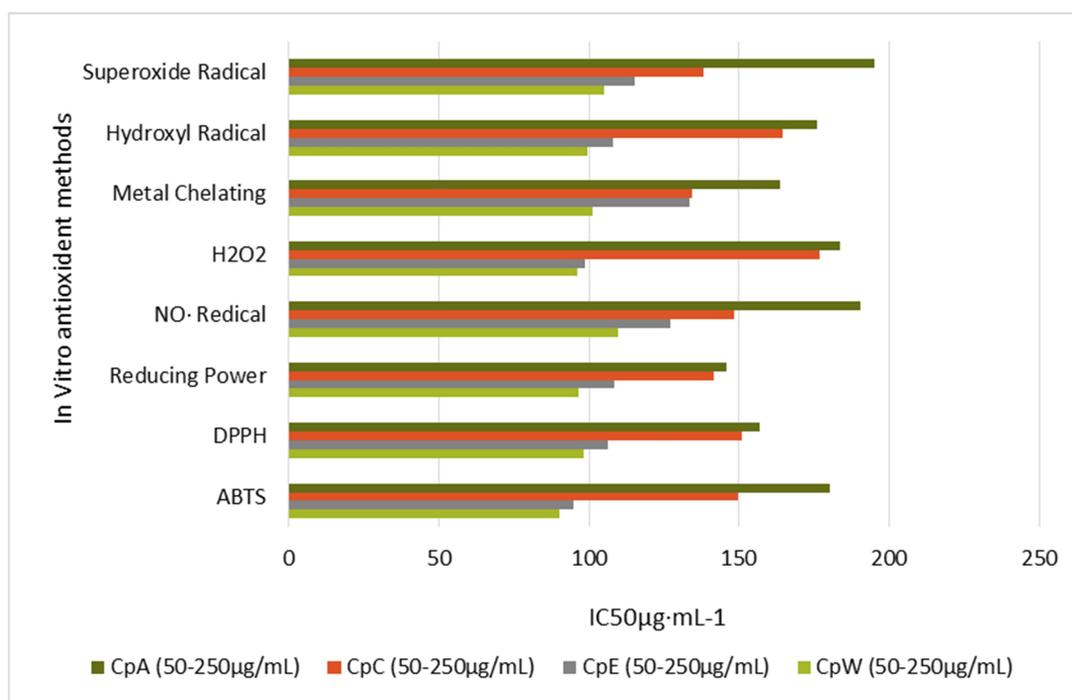


Figure 5: Comparison of antioxidant potential of various Cp extracts

CONCLUSION:

The outcome from varied in vitro antioxidants models demonstrated that the aqueous (CpW), ethanol (CpE) fraction of *Celastrus paniculatus* has a remarkable and noticeable antioxidant potential comparably to acetone (CpA) and chloroform (CpC) extract

because of the lesser IC₅₀ (µg·mL⁻¹) greater the antioxidant potential (Figure 5). The varied confirmative mechanisms for these various antioxidant schemes involve scavenging potential of superoxide anion radical, hydrogen-donating potential, reducing potential, NO[•], DPPH radical and

H₂O₂. The existence of various contrasting antioxidant components in water and ethanol extract of Cp are confirmed that control free radical scavenging potential and might be involve to reduce oxidative stress. Existent phytochemical works also enlighten various phytoconstituent in Cp of various geographical regions which can demonstrate potential antioxidant response. Therefore it is concluded that *Celastrus paniculatus* from the north-western Himalayas (Himachal Pradesh) can be the ideal and considerable source of natural antioxidants. The responses also validate the Ayurvedic literature of Indian system of medicine, as per Ayurveda *Celastrus paniculatus* seed oil is potent brain tonic. As per the current outcome, it can be recommend that *Celastrus paniculatus* can be employed to cure various neurological disorders like neurosarcoidosis.

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