

**Comparative Antioxidant Potential of Different  
Extracts of *Celastrus paniculatus* Willd. Seed**Fatema Tuz Zohera<sup>1</sup>, \*Md. Razibul Habib<sup>2</sup>, Mohammad Zafar Imam<sup>3</sup>,  
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**ABSTRACT**

The objective of the present study was to evaluate the comparative antioxidant potential of methanol, ethyl acetate, pet ether and water extracts of *Celastrus paniculatus* seed. Antioxidant activity was evaluated by using total phenol and flavonoid content determination assays, total antioxidant capacity, 1,1-diphenyl-2-picryl-hydrazil (DPPH) free radical assay, Reducing power assessment, Nitric oxide (NO) scavenging assay and Cupric ion reducing capacity assay (CUPRAC method). The extracts showed moderate antioxidant activity in a dose dependent manner. The extracts were found to contain phenolics and flavonoid compounds. In DPPH radical scavenging assay, ethyl acetate extract had the lowest IC<sub>50</sub> value (585.58µg/ml) compared to ascorbic acid. In nitric oxide scavenging assay IC<sub>50</sub> value was found to be 122.99µg/ml, 320.54µg/ml, 601.81µg/ml and 206.37µg/ml respectively for the Water, Methanol, Ethyl Acetate and Pet Ether extracts compared to 6.83µg/ml which was the IC<sub>50</sub> value for the reference ascorbic acid. The extracts also showed good reducing power. The results of the present study indicate that the extracts possesses significant antioxidant potential of which ethyl acetate extract is the most promising one and possess highest antioxidant potential.

**Key Words:** *Celastrus paniculatus*, antioxidant, DPPH, NO scavenging, CUPRAC, ROS.**INTRODUCTION**

Organisms have self defense mechanisms to protect themselves from the free radicals attack such as preventive antioxidant system that reduces the rate of free radical formation, and another is system to produce chain-breaking antioxidants that scavenge and stabilize free radicals. If free radical production rate exceeds the normal capacity of the antioxidant defense mechanisms, substantial tissue injury results (Rahman and Moon, 2007). Continuous exposure to chemicals and contaminants may lead to an increase in the amount of free radicals (HO•, O<sup>2•-</sup>, NO•) in the body beyond its capacity to control them and may lead to irreversible oxidative damage which includes biological damage, DNA damage, diabetes, respiratory tract disorders, carcinogenesis and cellular degeneration related to aging (Anderson et al., 2000; Tseng et al., 1997). In recent years the interest in natural antioxidants, especially of plant origin, has greatly increased as the possibility of toxicity of synthetic antioxidants (Jayaprakash and Rao, 2000).

*Celastrus paniculatus* (Family: Celastraceae) is known as Malkanjri in Bengali while in English known as Black-oil tree, is a woody climbing shrub. This plant has a remarkable reputation in the treatment of cognitive dysfunction, epilepsy, insomnia, rheumatism, gout, dyspepsia (Russo et al., 2001), edema, stomach disorders, nervous system disorders and as a brain tonic. Seeds of this plant are used in paralysis, leprosy, gout, rheumatism and sores (Kirtikar and Basu, 1987; Nadkarni, 1976). A sesquiterpene ester called Malkangunin (Lotter et al., 1978) sesquiterpene alkaloids named celapanin and celapagin have been isolated from the plant (Wagner et al., 1974; Wagner et al., 1975). The seed oil obtained from *C. paniculatus* has been used in Ayurvedic medicine for the treatment of several complaints including intestinal disorders and muscle relaxation (Borrelli et al., 2004). Seed oil of *C. paniculatus* has been reported to improve memory and the methanolic extract exhibits free-radical-scavenging properties and anti-oxidant

effects in human non-immortalized fibroblasts (Godkar et al., 2006). The seed of this plant has been found to enhance cognition and the mechanism by which it improves cognition is partly attributed to the antioxidant properties (Kumar and Gupta, 2002). In the present study we attempted to evaluate the comparative antioxidant potential of methanol, ethyl acetate, pet ether and water extracts of *Celastrus paniculatus* seed by different *in vitro* tests.

## **MATERIALS AND METHODS**

### **Collection of plant material and extraction**

The seed sample of *C. paniculatus* was collected from the local market of Dhaka, Bangladesh. The plant was identified by the experts of Department of Botany, Jahangirnagar University, Savar, Dhaka, Bangladesh and a voucher specimen is deposited there for further reference. The seeds of the plant was washed, cut and sliced and then dried under the sun and finally at 55°C in a mechanical dryer. The dried samples were ground to coarse powder with a mechanical grinder and then were subjected to extraction by Water, Methanol, Ethyl Acetate and Petroleum ether with a volume of 800ml for 3 days for allowing total extraction process.

### **Chemicals and drugs**

DPPH (1, 1-diphenyl, 2-picrylhydrazyl), TCA (trichloroacetic acid) and ferric chloride were obtained from Sigma Chemical Co. USA; Ascorbic acid was from SD Fine Chem. Ltd. India, ammonium molybdate from Merck, Germany.

### **Phytochemical screening**

The presences of different chemical constituents were identified by the characteristic color changes using standard procedures. Freshly prepared crude extracts of *C. paniculatus* were qualitatively tested for the presence of chemical constituents using the following reagents and chemicals: Alkaloids with Dragendorff's reagent, flavonoids with the use of Mg and HCl; tannins with ferric chloride and potassium dichromate solutions and saponins by the ability to produce stable foam after shaking and steroids with Libermann Burchard reagent, reducing sugars with Benedict's reagent and observed color change in respective cases (Ghani, 2003).

### **Determination of total phenolic content**

The total phenolic content of extracts was determined using Folin-Ciocalteu method (Singelton et al., 1999). The extracts were oxidized with Folin-Ciocalteu reagent and were neutralized with sodium carbonate. The absorbance of the resulting blue color solution was measured at 760nm after 60min using gallic acid (GA) as standard. Total phenolic content was expressed as mg GA equivalent/gm of extract.

### **Determination of total flavonoid content**

The flavonoid content was determined using a method described by Kumaran and Karunakaran (2007) using quercetin as a reference compound. 1 mg of plant extract in methanol was mixed with 1ml aluminium trichloride in Ethanol (20mg/ml) and a drop of acetic acid, and then diluted with Ethanol. The absorption at 415nm was read after 40 min. The absorption of blank samples and standard quercetin solution (0.5mg/ml) in methanol was measured under the same conditions.

### **Determination of total antioxidant capacity**

The antioxidant activity of the extracts of *C. paniculatus* was evaluated by the phosphomolybdenum method as described by Prieto et al. (1999). The assay is based on the reduction of Mo (VI)-Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acidic pH. 0.3ml extract was combined with 3ml of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90min. Then the absorbance of the solution was measured at 695nm using a spectrophotometer (Shimadzu, UV-150-02) against blank after cooling to room temperature. Methanol (0.3ml) in the place of extract is used as the blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid.

### **DPPH radical scavenging activity**

The free radical scavenging capacity of the extracts was determined using DPPH (Hasan et al., 2006; Alam et al., 2008). A methanol DPPH solution (0.004% w/v) was mixed with solutions of different concentrations (0 to 500µg) of *C. paniculatus* extracts and after 10 min the absorbance

was read at 515nm using a spectrophotometer. Ascorbic acid was used as a standard. The inhibition curve was plotted and IC<sub>50</sub> values were calculated.

### Reducing power

The reducing power of *C. paniculatus* extractives was determined according to the method described by Oyaizu (1986). Different concentrations of *C. paniculatus* extracts in 1ml of distilled water was mixed with phosphate buffer (2.5ml, 0.2M, pH 6.6) and potassium ferricyanide [K<sub>3</sub>Fe(CN)<sub>6</sub>] (2.5ml, 1%). The mixture was incubated at 50°C for 20min. A portion (2.5ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3,000rpm for 10min. The upper layer of the solution (2.5ml) was mixed with distilled water (2.5ml) and FeCl<sub>3</sub> (0.5ml, 0.1%) and the absorbance was measured at 700nm. Ascorbic acid was used as a reference standard. Phosphate buffer (pH) was used as blank solution.

### Nitric oxide scavenging assay

Nitric oxide radical scavenging capacity was estimated on the basis of Griess Illosvoy reaction using method followed by Govindarajan et al. (2003). In this investigation, Griess-Illosvoy reagent was modified by using naphthyl ethylene diamine dihydrochloride (0.1% w/v) instead of 1-naphthylamine (5%). The reaction mixture (3ml) containing sodium nitroprusside (10mM, 2ml), phosphate buffer saline (0.5ml) and *C. paniculatus* extracts (5 to 200µg/ml) or standard solution (ascorbic acid, 0.5ml) was incubated at 25°C for 150min. After incubation, 0.5ml of the reaction mixture mixed with 1ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5min for completing diazotization. Then, 1ml of naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30min at 25°C. A pink colored chromophore formed in diffused light. The absorbance of these solutions was measured at 540nm against the corresponding blank solutions.

### Cupric Reducing Antioxidant Capacity (CUPRAC)

Cupric ion reducing capacity was determined according to the method described by Resat et al. (2004). Where CuCl<sub>2</sub>.2H<sub>2</sub>O solution, ammonium acetate buffer (pH 7.0), neocuproin solution and distilled water were added to *C. paniculatus* extracts to adjust the final volume to 4.1ml. The mixture was incubated for 1 hour at room temperature and absorbance was measured at 450nm using a spectrophotometer against blank. Ascorbic acid was used as a standard.

## RESULTS

### Phytochemical screening

The results of phytochemical screening of the crude extracts (Table 1) reveal the presence of carbohydrate in all extracts. Glycoside, saponin, flavonoids, alkaloids and tannins were present.

**Table 1: Result of phytochemical screening of different extracts of the seed of *C. paniculatus*.**

Tests	CPWT	CPME	CPEA	CPPE
Carbohydrate	+	+	+	+
Glycoside	-	+	-	-
Saponin	+	+	-	-
Steroid	+	-	-	-
Alkaloid	+	+	+	-
Glucoside	+	-	+	-
Tannin	-	+	+	-
Flavonoid	+	+	+	-

CPWT, CPME, CPEA and CPPE denote for Water, Methanol, Ethyl Acetate and Petroleum ether extracts respectively of *C. paniculatus* seed. (+): Present; (-): Absent

### Total phenol, total flavonoid and total antioxidant activity

Table 2 shows the results of total phenol and flavonoid content and total antioxidant capacity of the seed extracts of *C. paniculatus*. The total phenol, total flavonoid content and total antioxidant capacity of the extracts were expressed in gallic acid, quercetin and ascorbic acid equivalents respectively. The content of phenolics in the extracts under this investigation correlates with the antioxidant activity; being highest in Ethyl Acetate extract (32.62 mg/g GAE), Methanolic extract showed moderate results (15.79 mg/g GAE). Among the extracts highest amount of flavonoid was

found in methanolic extract (12.53 mg/g quercetin equivalent) while Water and Methanolic fraction exhibited the most prominent and similar result for total antioxidant capacity.

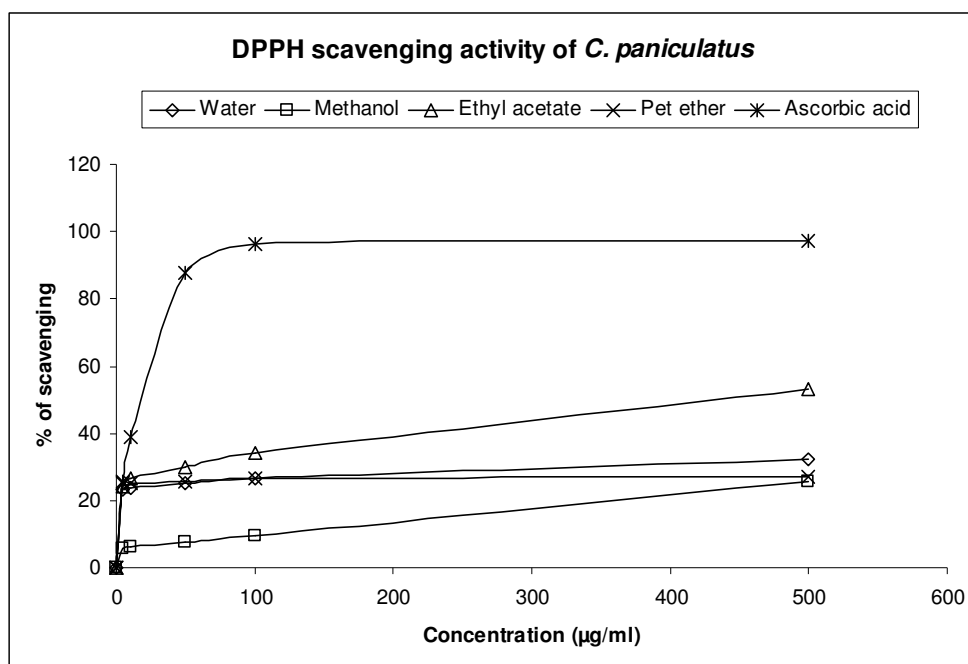
**Table 2: Total amount of plant phenolics, flavonoid content and total antioxidant capacity of *C. paniculatus*.**

Extracts	Total phenol (In mg/g , Gallic acid equivalents)	Total flavonoid (In mg/g, quercetin Equivalents)	Total antioxidant (In mg/g, ascorbic acid equivalents)
CPWT	7.13	10.56	100.88
CPME	15.79	12.53	100.16
CPEA	32.62	4.23	117.2
CPPE	9.28	1.02	68.72

CPWT, CPME, CPEA and CPPE denote for Water, Methanol, Ethyl Acetate and Petroleum ether extracts respectively of *C. paniculatus* seed.

**DPPH radical scavenging activity**

The DPPH radical scavenging activity of *C. paniculatus* is shown in Figure 1. This activity was found to increase with increasing concentration of the extracts. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515-517nm and also for a visible deep purple color. The IC<sub>50</sub> value of the ethyl acetate extract was 585.58µg/ml while the IC<sub>50</sub> value of ascorbic acid was 11.24µg/ml. The IC<sub>50</sub> value of the water, methanol and pet ether extracts were found to be >10,000µg/ml.



**Figure 1: DPPH radical scavenging activity of different extract of *C. paniculatus*.**

**Reducing power**

Figure 2 represents the reductive capabilities of the plant extracts compared to Ascorbic acid which was determined using the potassium ferricyanide reduction method. The reducing power of the extracts was moderately strong while increasing dose it shows little increment. However, Ethyl Acetate extract displayed the highest reducing power followed by methanol and water extracts.

**Nitric oxide scavenging capacity**

The different extracts of the *C. paniculatus* exhibited moderate scavenging of nitric oxide (Figure 3) with an IC<sub>50</sub> value of 122.99µg/ml, 320.54µg/ml, 601.81µg/ml and 206.37µg/ml respectively for the Water, Methanol, Ethyl Acetate and Pet Ether extracts compared to 6.83µg/ml which was the IC<sub>50</sub> value of ascorbic acid. All of the plant extracts showed NO scavenging activity but among the different extractives, Water extract possesses highest NO scavenging capacity.

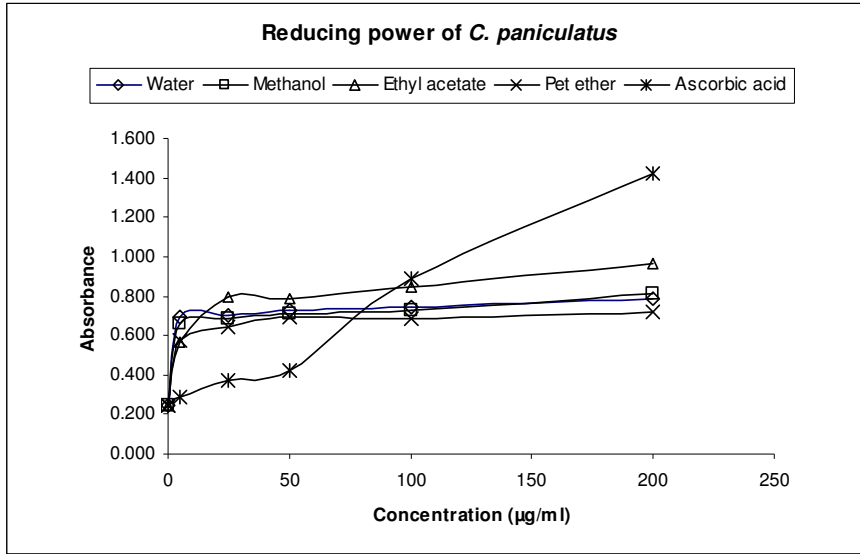


Figure 2: Reducing power of the crude plant extracts of *C. paniculatus* and ascorbic acid.

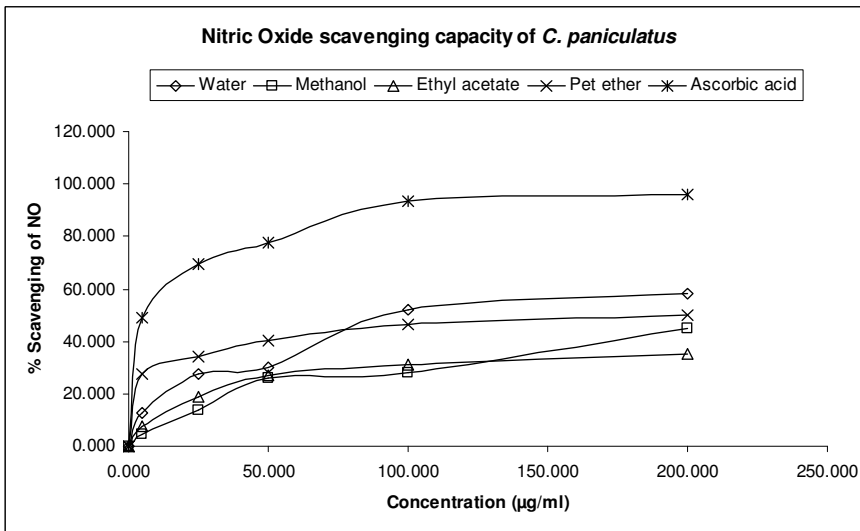


Figure 3: Nitric oxide scavenging activity of different extracts of *C. paniculatus* and ascorbic acid.

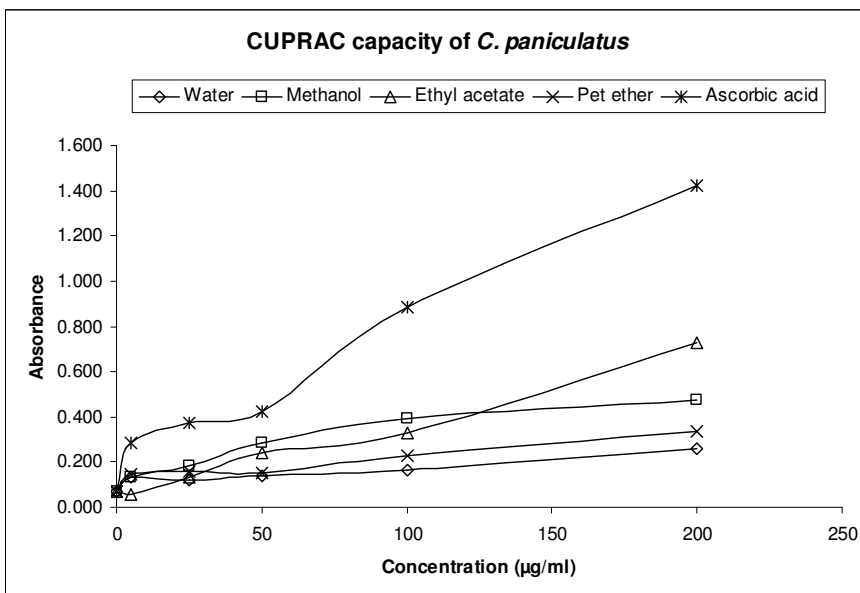


Figure 4: Comparative Cupric reducing antioxidant capacity of different extracts of *C. paniculatus*.

### Cupric Reducing Antioxidant Capacity

The different extracts of the *C. paniculatus* showed dose dependent reducing capacity where Ethyl Acetate showed very good activity and water extracts showed minimum activity in terms of cupric ion reducing antioxidant potential and shown in Figure 4.

### DISCUSSION

Different studies suggest that different types of polyphenolic compounds (flavonoids, phenolic acids) found in plants have multiple biological effects, including antioxidant activity (Vinson et al., 1995) and present studies indicate the presence of polyphenolic compounds in different extracts of *C. paniculatus*. Additionally, it has been determined that the antioxidant effect of plant products is mainly due to radical scavenging activity of phenolic compounds such as flavonoids, polyphenols, tannins, and phenolic terpenes (Rahman and Moon, 2007). In DPPH test, which is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action. Ethyl Acetate extract of *C. paniculatus* showed good DPPH scavenging activity. Ascorbic acid was chosen as the reference antioxidant for this test. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515-517nm and also for a visible deep purple color. NO scavenging capacity of the extracts may help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to the human health. Nitric oxide is also implicated for inflammation, cancer and other pathological conditions (Moncada et al., 1991). The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Duh et al., 1999).

### CONCLUSION

Considering the results of the conducted study, it can be implicit that the seed of *Celastrus paniculatus* possess moderate antioxidant potential which correlates traditional use in various diseases conditions as well as with various established research reports. However, the study conducted here are preliminary in nature and there are plenty of scopes for further *in vivo* pharmacological investigations followed by compound isolation which can result in development of a new lead compound for drug discovery.

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