



E-ISSN: 2278-4136  
P-ISSN: 2349-8234  
JPP 2018; 7(4): 1596-1599  
Received: 04-05-2018  
Accepted: 08-06-2018

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## Assessment of *In-vitro* antioxidant activity of various bark extracts of *Crateva magna* (Lour) DC. (Capparaceae)

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### Abstract

Antioxidant effects of the Petroleum ether, Benzene, ethyl acetate, Methanol and Ethanol extracts of the bark of *Crateva magna* were tested on the basis of scavenging activity of the free radical DPPH (1, 1-diphenyl-2-picryl-hydrazyl), Hydroxyl, ABTS, Superoxide and reducing power assays. The methanol extract of *C. magna* showed the highest antioxidant activity and the activity of the extract increased with the increasing concentrations. All the analysis was made with the use of UV-Visible Spectrophotometer.

**Keywords:** ABTS, antioxidant, free radicals, medicinal plant, DPPH

### Introduction

Free radicals are persistently generated resulting in extensive damage to tissues and biomolecules leading to form the various diseases. Medicinal plants are good sources with antioxidant property are employed as an alternative medicine to mitigate the diseases associated with oxidative stress [1, 2]. Antioxidants are widely used in food supplements and have been investigated for the preventable of diseases such as cancer, coronary heart disease and even altitude sickness. Antioxidants or inhibitors of oxidation are compounds which retard or prevent the oxidation and in general prolong the life of the oxidizable matter [3]. Medicinal plant parts are commonly rich in phenolic compounds, such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins. These compounds have multiple biological effects including antioxidant activity [4].

A well-known plant in herbal world for its extensive range of application in medicinal reasons is the plant *Crateva magna* which belongs to the family Capparaceae. The leaves are deciduous three foliolate; petioles 3.8-7.6 cm long; leaflets 5-15 ovate, lanceolate or obovate, acute or acuminate, attenuate at the base, entire, glabrous on both surfaces, pale beneath, and reticulately veined [5]. It is used as an anti-spasmodic, hypotensive, anti-inflammatory, hypoglycemic, anti protozoal, Anthelmintic, analgesic purposes. In folk medicine, its stem pith is used for lactation after child birth, treat urinary disorders, kidney bladder stones, fever, vomiting and gastric irritation by the ethnic peoples of Kandhamal district of Orissa known as Eastern Ghats of India [6, 7, 8]. The major constituent is the Triterpines, which has been shown to have these various activities. Other constituents are the alkaloids, minor flavonoides, sterols, Triterpines and the isothiocyanate glucosides. The present study, the crude extracts of *C. magna* were investigated for their antioxidant potential of super oxide radical scavenging activity, ABTS radical scavenging activity, DPPH scavenging activity, Hydroxyl radical & reducing power assays.

### Materials and Methods

#### Collection of plants

The fresh plant parts (bark) of *Crateva magna* were collected from Vellamadam, Nagercoil District, Tamil Nadu, India. The gathered samples were cut into small pieces and shade dried until the fracture is identical and even. The dried plant material was crushed or grinded by using a blender and separated to get uniform particles by using sieve No. 60. The final uniform powder was used for the extraction of active constituents of the plant material.

#### Preparation of extract

100 g of the coarse powder of bark of *C. magna* was extracted successively with 250 ml of alcoholic and organic solvents (Petroleum ether, Benzene, ethyl acetate, Methanol and Ethanol) in a Soxhlet apparatus for 24 hrs. All the extracts were filtered through Whatman No.

41 filter paper separately and all the extracts were concentrated in a rotary evaporator. All the concentrated extracts were subjected for *in vitro* antioxidant activity.

### Antioxidant activity

#### DPPH radical scavenging activity

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant component. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH-H<sup>[9]</sup>. The free radical scavenging activity of all the extracts was evaluated by 1, 1-diphenyl 1-2-picryl-hydrazyl (DPPH) according to the previously reported method (Blois, 1958). Briefly, 0.1 mM solution of DPPH in methanol was prepared and 1 ml of this solution was added to 3 ml of the solution of all extracts at different concentrations (50, 100, 200, 400 and 800 µg/ml). The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer (Genesys 10s UV: Thermo electron corporation). Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated by using the following formula. DPPH scavenging effect (% inhibition) =  $(A_0 - A_1) / A_0 \times 100$  Where, A<sub>0</sub> is the absorbance of the control and A<sub>1</sub> is the absorbance of the test samples and reference. All the tests were performed in triplicates and the results were averaged.

#### Hydroxyl radical scavenging activity

The scavenging capacity for hydroxyl radical was measured according to the modified method of Halliwell *et al.*<sup>[10]</sup>. Stock solutions of EDTA (1 mM), FeCl<sub>3</sub> (10 mM), Ascorbic Acid (1 mM), H<sub>2</sub>O<sub>2</sub> (10 mM) and Deoxyribose (10 mM) were prepared in distilled deionized water. The assay was performed by adding 0.1 ml EDTA, 0.01 ml of FeCl<sub>3</sub>, 0.1 ml H<sub>2</sub>O<sub>2</sub>, 0.36 ml of deoxyribose, 1.0 ml of the extract of different concentration (50, 100, 200, 400 & 800 µg/ml) dissolved in distilled water, 0.33 ml of phosphate buffer (50 mM, pH 7.9), 0.1 ml of ascorbic acid in sequence. The mixture was then incubated at 37 °C for 1 hour. 1.0 ml of the incubated mixture was mixed with 1.0 ml of 10% TCA and 1.0 ml of 0.5% TBA (in 0.025 M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532 nm. The hydroxyl radical scavenging activity of the extract is reported as % inhibition of deoxyribose. The degradation is calculated by using the following equation

$$\text{Hydroxyl radical scavenging activity} = (A_0 - A_1) / A_0 \times 100$$

Where, A<sub>0</sub> is the absorbance of the control and A<sub>1</sub> is the absorbance of the test samples and reference. All the tests were performed in triplicates and the results were averaged.

#### Superoxide radical scavenging activity

The superoxide anion scavenging activity was measured as described by Srinivasan *et al.*<sup>[11]</sup>. The superoxide anion radicals were generated in 3.0 ml of Tris-HCl buffer (16 mM, pH 8.0) containing 0.5 ml of NBT (0.3 mM), 0.5 ml NADH (0.936 mM) solution, 1.0 ml extract of different concentrations (50, 100, 200, 400 & 800 µg/ml) and 0.5 ml Tris-HCl buffer (16 mM, pH 8.0). The reaction was started by adding 0.5 ml PMS solution (0.12 mM) to the mixture, incubated at 25 °C for 5 min and the absorbance was

measured at 560nm against a blank sample, ascorbic acid. The percentage inhibition was calculated by using the following equation Superoxide radical scavenging activity =  $(A_0 - A_1) / A_0 \times 100$  Where, A<sub>0</sub> is the absorbance of the control and A<sub>1</sub> is the absorbance of the test samples and reference. All the tests were performed in triplicates and the results were averaged.

#### Antioxidant activity by radical cation (ABTS+)

ABTS assay was based on the slightly modified method of Huang *et al.*<sup>[12]</sup>. ABTS radical cation (ABTS+) was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12-16 hrs before use. The ABTS+ Solution were diluted with ethanol to an absorbance of 0.70 + 0.02 at 734 nm. After addition of 100 µL of sample or trolox standard to 3.9 ml of diluted ABTS+ solution, absorbance was measured at 734 nm by Genesys 10S UV-VIS (Thermo scientific) exactly after 6 minutes. Results were expressed as trolox equivalent antioxidant capacity (TEAC).

$$\text{ABTS radical cation activity} = (A_0 - A_1) / A_0 \times 100$$

Where, A<sub>0</sub> is the absorbance of the control and A<sub>1</sub> is the absorbance of the test samples and reference. All the tests were performed in triplicates and the results were averaged.

#### Reducing power

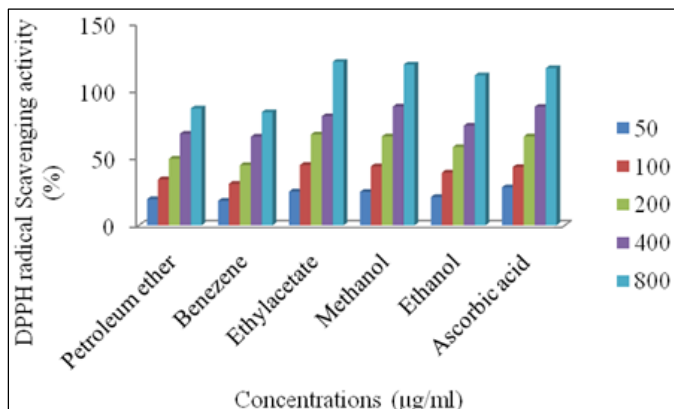
The reducing power of the extract was determined by the method of Kumar and Hemalatha<sup>[13]</sup>. 1.0 ml of solution containing 50, 100, 200, 400 & 800 µg/ml of extract was mixed with sodium phosphate buffer (5.0 ml, 0.2 M, pH 6.6) and potassium ferricyanide (5.0 ml, 1.0%). The mixture was incubated at 50 °C for 20 minutes. Then 5ml of 10% trichloroacetic acid was added and centrifuged at 980 g (10 minutes at 5 °C) in a refrigerated centrifuge. The upper layer of the solution (5.0 ml) was diluted with 5.0 ml of distilled water and ferric chloride and absorbance read at 700 nm. The experiment was performed thrice and results were averaged.

### Results and Discussion

Medicinal plants rich in phenolics are increasingly being used in the food industry because they retard oxidative degradation of lipids and improve the quality and nutritional value of food. In the present study, antioxidant capacity of various solvents of *C. magna* bark extracts was examined using five different assays.

#### DPPH radical scavenging activity

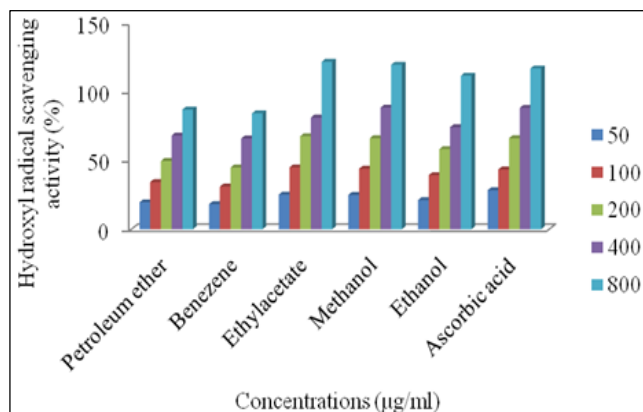
DPPH is a protonated radical having the characteristic absorption maxima at 517 nm which decreases with the scavenging of the proton radical by natural plant extracts. Hence, DPPH finds applications in the determination of the radical scavenging activity of plant materials<sup>[14]</sup>. DPPH radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of bark of *C. magna* are shown in figure 1. The scavenging effect of ascorbic acid, the standard and various solvent extracts studied increases with the increase in the concentration. Among the solvent tested, ethanol extract of bark of *C. magna* exhibited the highest DPPH radical scavenging activity. At 800µg/ml concentration, ethanol extract of leaf of *C. magna* possessed 116.16%. The concentration of *C. magna* bark ethanol extract needed for 50% inhibition (IC<sub>50</sub>) was 30.16 mg/ml, while ascorbic acid needed 28.54 mg/ml (Table 1).



**Fig 1:** Effect of different solvent extract of *C.magna* bark on DPPH assay

### Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of bark of *C. magna* are shown in figure 2. The methanol extract of *C. magna* bark showed a very potent hydroxyl radical scavenging activity. At 800µg/ml concentration, *C. magna* leaf possessed 109.56% hydroxyl radical scavenging activity. The concentration of *C. magna* bark methanol extract needed for 50% inhibition ( $IC_{50}$ ) was found to be 28.93 mg/ml, whereas 29.56 mg/ml (Table 1) needed for ascorbic acid. The hydroxyl radical scavenging ability of the extracts was determined by its ability to compete with deoxyribose for hydroxyl radical. The crude extracts (methanol and ethanol) of *C. magna* extract compete with deoxyribose and diminish chromogen formation in a dose dependant manner. In this assay, 2-deoxy-2-ribose was oxidized when exposed to hydroxyl radicals generated by Fenton-type reaction. The oxidative degradation can be detected by heating the products with TBA under acid conditions to develop a pink chromogen with a maximum absorbance at 532 nm <sup>[15]</sup>.

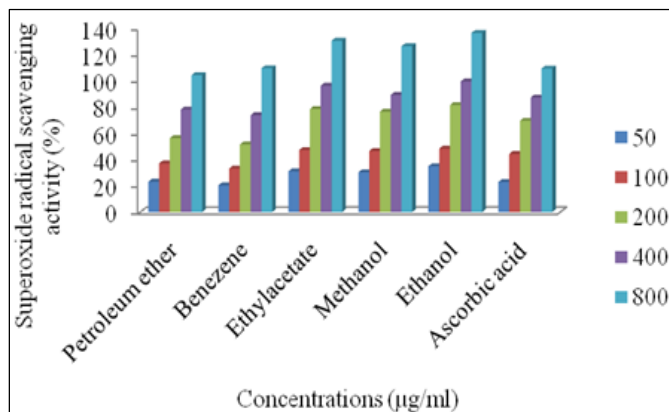


**Fig 2:** Effect of different solvent extract of *C. magna* bark on Hydroxyl assay

### Superoxide anion radical scavenging activity

All the extracts of *C. magna* were subjected to be superoxide radical scavenging activity and the results are shown in figure 3. The ethanol extract of bark of *C. magna* exhibited the maximum superoxide radical scavenging activity 136.54% at 800µg/ml concentration. This scavenging activity was higher than that of ascorbic acid, the standard

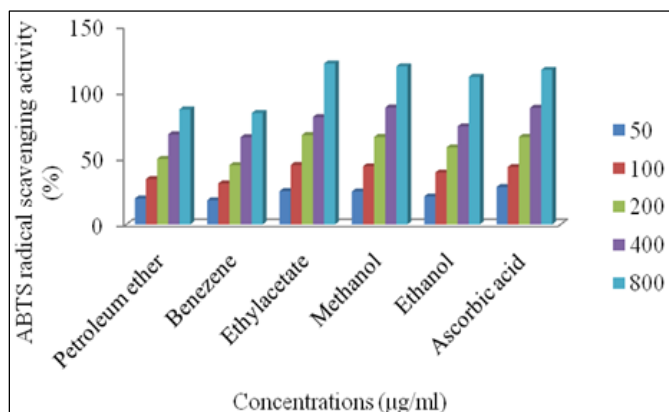
which had 109.36% scavenging activity. The  $IC_{50}$  value of ethanol extract of *C. magna* bark on superoxide radical was found to be 35.22 mg/ml and 29.31 mg/ml for ascorbic acid, respectively (Table 1). The ethanolic extract was found to be an effective scavenger of superoxide radical generated by photo reduction of riboflavin. Superoxide anion radical is one of the strongest Reactive Oxygen Species among the free radicals and get converted to other harmful reactive oxygen species such as hydrogen peroxide and hydroxyl radical, damaging biomolecules which results in chronic diseases <sup>[16]</sup>.



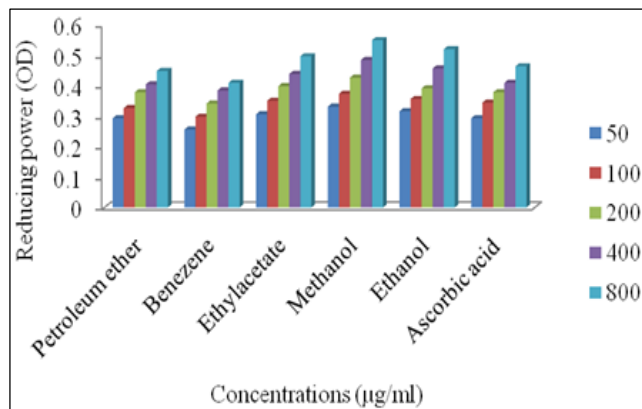
**Fig 3:** Effect of different solvent extract of *C.magna* bark on Superoxide anion assay

### ABTS radical cation scavenging activity

The different solvent extracts of *C. magna* bark were subjected to be ABTS radical cation scavenging activity and the results are shown in figure 4. The methanol extract of *C. magna* bark exhibited potent ABTS radical cation scavenging activity in concentration dependent manner. At 800µg/ml concentration, the methanol extract of *C. magna* bark possessed 119.56%. The quantity of *C. magna* methanol extract required to produce 50% inhibition of ABTS radical 29.11 mg/ml whereas 30.13 mg/ml (Table 1) needed for trolox. ABTS radical cation scavenging activity respectively. ABTS radical cation scavenging assay involves a method that generates a blue/green ABTS chromophore via the reaction of ABTS and potassium persulfate. The ABTS radical cation is generated by the oxidation of ABTS with potassium persulfate, its reduction in the presence of hydrogen donating antioxidants is measured spectro photo metrically at 745 nm.



**Fig 4:** Effect of different solvent extract of *C.magna* bark on ABTS assay



**Fig 5:** Effect of different solvent extract of *C. magna* bark on reducing power assay

**Table 1:** IC<sub>50</sub> values of different solvent extracts of the leaf extracts of *C. magna*

Solvents	IC <sub>50</sub> (µg/ml)			
	DPPH	Hydroxyl	ABTS	Superoxide anion
P. ether	23.67	26.37	23.08	26.34
Benzene	25.16	20.16	22.56	28.98
Ethyl acetate	33.16	31.13	30.84	34.67
Methanol	29.22	28.93	29.11	32.84
Ethanol	30.16	24.16	28.54	35.22
Ascorbic acid	28.54	29.56	-	29.31
Trolox	-	-	30.13	-

### Reducing Power

Table 1 showed the reducing abilities of different solvent extracts of *C. magna* bark were compared to the standard ascorbic acid. Absorbances of the extracts were increased with the concentration. A higher absorbance indicated a higher reducing power. Among the solvent tested, the methanol extracts of bark of *C. magna* exhibited higher reducing activity. This result indicates that the extracts may consist of polyphenolic compounds that usually show great reducing power. This has been justified by methanol extract being the most reducing agent with highest phenolic content. In this assay, Fe (III) reduction is often used as a significant indicator of electron donating activity which is an important mechanism of phenolic antioxidant action and is correlated with the presence of reductones which exhibits its antioxidant action by breaking the radical chain by donating a hydrogen atom [17].

The results of the present study revealed that *C. magna* bark extracts possessed potent free radical scavenging ability in methanol and ethanol extracts. The activity observed may be attributed to the presence of phenolic and flavanoid contents in the methanol and ethanol extracts and further we conclude that this plant is a potential candidate for natural antioxidant.

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