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Research Article

EVALUATION OF ANTIOXIDANT ACTIVITY OF LEAF EXTRACTS OF *CRATEVA MAGNA* (LOUR) DC. (CAPPARACEAE)

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A B S T R A C T

The present study was to compare the effects of in-vitro antioxidant activity between the various leaf extracts (Petroleum ether, Benzene, Ethyl acetate, Methanol and Ethanol) of *Crateva magna*. The free radical scavenging activity was found to be high in ethanol and methanol extracts for DPPH, hydroxyl, superoxide, ABTS and reducing power assays in a concentration dependent manner followed by ethyl acetate, petroleum ether and benzene extracts. The present results recommend that the leaf extracts (methanol and ethanol) of *C.magna* could serve as potential alternative source antioxidants and can be explored as a good therapeutic agent in the free radical induced diseases.

INTRODUCTION

There has been increasing interest in natural antioxidants found in medicinal plants because of the carcinogenic effects of synthetic antioxidants (Sasaki *et al*., 2002). Oxidative stress causes collapse of the mitochondrial membrane potential, which is associated with many age-related diseases (Salminen *et al*., 2012; Facecchia *et al*., 2011). Dietary antioxidants, such as vitamin C and phenolic compounds, present in foods contribute to defense against oxidative stress (Baeza *et al*., 2014; Dominguez-Perles *et al*., 2014). Overall, natural antioxidants can protect the human body from free radicals and retard the lipid oxidative rancidity in foods. The mechanism of antioxidants includes the suppression of ROS formation, the hindrance of enzymes or chelating of elements engaged in free-radical production. Moreover, antioxidants scavenge reactive species, and unregulated antioxidant defences.

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 as deciduous three foliolate; leaflets 5–15 ovate, petioles 3.8–7.6 cm lengthy; lanceolate or obovate, acute or acuminate, soothe at the bottom, glabrous on both the surfaces, pale beneath, and reticulately veined (Inayathulla *et al*., 2010).

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It is employed as an anti spasmodic, anti-inflammatory, hypotensive, hypoglycemic, anti protozoal, Anthelmintic, analgesic reasons. 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 (Sovan Pattanaik *et al*., 2012; Gagandeep and Kalidhar, 2006; Kritikar and Basu, 2005). The most important constituent is the Triterpines, which has been exposed to have these different activities. Other constituents are the alkaloids, minor flavonoides, Triterpines, sterols and the isothiocyanate glucosides. The present work was aimed to determine the in-vitro free radical scavenging activity between the leaf extracts of *Crateva magna*.

MATERIALS AND METHODS

Collection of plants

The fresh plant parts (leaf) of *Crateva magna* were collected from Vellamadam, Nagercoil, Kanayakumari 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 leaf of *C. magna* was extracted successively with 250 ml of alcoholic and organic solvents (Peroleum 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 constant free radical and is widely used to calculate the radical scavenging activity of antioxidant component. This process is based on the reduction of DPPH in methanol solution in the company of a hydrogen donating antioxidant because of the formation of the non-radical form DPPH-H (Blois, 1958). By 1, 1- diphenyl-2-picryl-hydrazyl (DPPH) the free radical scavenging action of all the extracts was assessed as per the previously reported method (Blois, 1958). 0.1 mM solution of DPPH in methanol was prepared. 1 ml of this solution was added to 3 ml of the solution of all extracts at dissimilar concentrations (50, 100, 200, 400 and 800 µg/ml). The mixtures were shaken vigorously. This was allowed to stand at room temperature for 30 minutes. Then the absorbance was calculated at 517 nm using a UV-VIS spectrophotometer (Genesys 10s UV: Thermo electron corporation). Ascorbic acid was utilized as the reference. Lower absorbance values of reaction mixture specify higher free radical scavenging action. The capacity to scavenge the DPPH radical was computed by using the succeeding formula.
DPPH scavenging effect (% inhibition) = (A0 - A1) / A0 X 100

where, A0 is the absorbance of the control and A1 is the absorbance of the test samples and reference. All the tests were executed in triplicates and the end results were averaged.

Hydroxyl radical scavenging activity

As per the modified method of Halliwell *et al.* (1987) the scavenging ability for hydroxyl radical was estimated. Using dilute deionized water, Stock solutions of EDTA (1 mM), $FeCl_3$ (10 mM), H2O2 (10 mM), Ascorbic Acid (1 mM), and Deoxyribose (10 mM) were made. The assay was performed by adding 0.1 ml EDTA, 0.36 ml of deoxyribose 0.01 ml of FeCl3, 0.1 ml H_2O_2, 1.0 ml of the extract of unlike 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_oC 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 action of the extract is reported as % inhibition of deoxyribose. The degradation is determined by using the subsequent equation

Hydroxyl radical scavenging activity = (A0 - A1) / A0 X 100
Where, A0 is the absorbance of the control and A1 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 computed as depicted by Srinivasan *et al.* (2007). 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^oC 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 = (A0 - A1) / A0 X 100
where, A0 is the absorbance of the control and A1 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.* (2011). ABTS radical cation (ABTS+) was made by reacting 7 mM ABTS solution with 2.45 mM potassium persulphate. This mixture is allowed to stand in the dark at room temperature for 12-16 hrs before use. The ABTS+ Solution were concentrated with ethanol to an absorbance of 0.70 + 0.02 at 734 nm. Subsequent to addition of 100 µL of sample or trolox standard to 3.9 ml of diluted ABTS+ solution, absorbance was worked out. This was done at 734 nm by Genesys 10S UV-VIS (Thermo scientific) precisely following 6 minutes. Results were expressed as trolox equivalent antioxidant capacity (TEAC).
ABTS radical cation activity = (A0 - A1) / A0 X 100
Where, A0 is the absorbance of the control and A1 is the absorbance of the test samples and reference. All the tests were executed in triplicates and the end results were averaged.

Reducing Power

The reducing power of the extract was decided using the method of Kumar and Hemalatha (2011). 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^o C for 20 minutes. Then 5ml of 10% trichloroacetic acid was added and centrifuged at 980 g (10 minutes at 5^oC) 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

In the present study, antioxidant capacity of various solvents of *C.magna* leaf extracts was examined using five different assays.

DPPH radical scavenging activity

DPPH radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of leaf 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 ark of *C.magna* exhibited the highest DPPH radical scavenging activity.

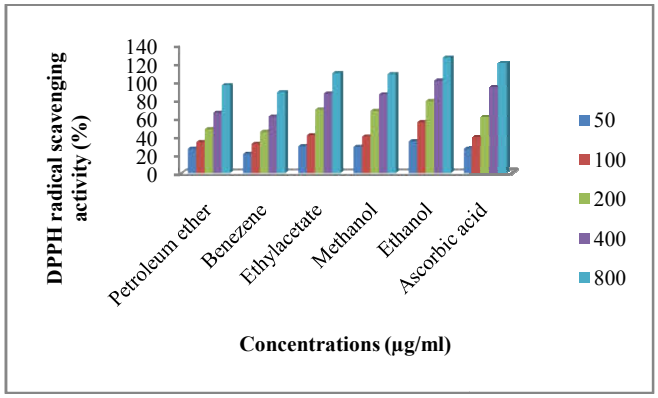

Figure 1 DPPH radical scavenging activity of various leaf extracts of *C.magna*

At 800µg/ml concentration, ethanol extract of leaf of *C.magna* possessed 126.30 % DPPH radical scavenging activity. The concentration of *C.magna* leaf ethanol extract needed for 50 % inhibition (IC_{50}) was 26.31 mg/ml, while ascorbic acid needed 28.54 mg/ml (Table 1).

Table 1 IC_{50} values of different solvent extracts of the leaf extracts of *C. magna*

Solvents	IC_{50} (µg/ml)			
	DPPH	Hydroxyl	ABTS	Superoxide anion
P.ether	31.92	28.06	36.21	34.88
Benzene	26.05	33.96	36.98	31.16
Ethyl acetate	24.65	26.15	29.36	27.38
Methanol	22.92	27.36	26.37	24.13
Ethanol	26.31	32.16	31.16	32.16
Ascorbic acid	28.54	29.56	-	29.31
Trolox	-	-	30.13	-

The DPPH free radical scavenging assay is a simple technique to assess antioxidant activity in a relative short time compared to the other methods (Koleva *et al*., 2002). DPPH is a relatively stable radical. The assay is based on the amount of the scavenging capability of the antioxidants to the stable radical DPPH which reacts with appropriate reducing agent. The electrons become paired off and solution loos its colour stoichiometrically depending on the number of electrons taken up. At wavelength 517nm the colour change can be quantified by its decrease of absorbance. The antioxidants exert their DPPH free radical scavenging due to their hydrogen donating ability (Kannat *et al*., 2007).

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of leaf of *C.magna* are shown in figure 2.

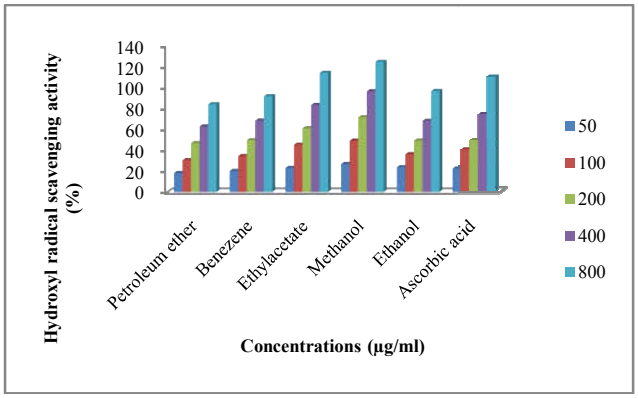

Figure 2 Hydroxyl radical scavenging activity of various leaf extracts of *C.magna*

The methanol extract of *C.magna* leaf showed a very potent hydroxyl radical scavenging activity. At 800µg/ml concentration, *C.magna* leaf possessed 124.38% hydroxyl radical scavenging activity. The concentration of *C.magna* leaf methanol extract needed for 50 % inhibition (IC_{50}) was found to be 27.36 mg/ml, whereas 29.56 mg/ml (Table 1) needed for ascorbic acid. The hydroxyl radical is one of the most reactive oxygen species in living systems. It damages the cell by reacting with the polyunsaturated fatty acid of cell membrane phospholipids (Halliwell, 2008). Thus, removing OH radicals is very important for the protection of biological systems. In this study all the crud extracts generally showed good hydroxyl radical scavenging activity in a concentration dependent manner.

Superoxide anion radical scavenging activity

Superoxide anion is a weak oxidant produced through a variety of biological reactions is highly toxic. The superoxide anion derived from dissolved oxygen from PMS/NADH coupling reaction reduces NBT in the PMS/NADH-NBT system. (Toda *et al*., 1988). Superoxide anion radical is known as an early radical. This plays an imperative role in the formation of other reactive oxygen-species, for example hydrogen peroxide or singlet oxygen. Superoxide is generated in vivo by several oxidative enzymes, including xanthine oxidase. A decrease in absorbance indicated the antioxidant activity of the extracts which may be due to the inactivation or consumption of superoxide anion radicals produced in the reaction mixture. All the extracts of *C.magna* leaves were subjected to be superoxide radical scavenging activity and the results are shown in figure 3.

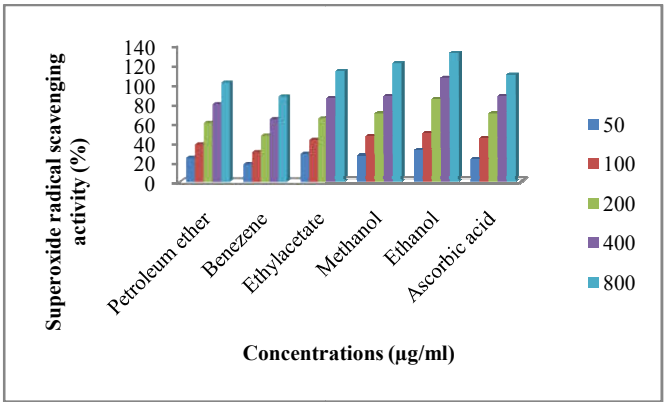

Figure 3 Superoxide anion scavenging activity of various leaf extracts of *C.magna*

The ethanol extract of leaf of *C.magna* exhibited the maximum superoxide radical scavenging activity 131.84% 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* leaf on superoxide radical was found to be 32.16 mg/ml and 29.31 mg/ml for ascorbic acid, respectively (Table 1).

ABTS radical cation scavenging activity

The different solvent extracts of *C.magna* leaf were subjected to be ABTS radical cation scavenging activity and the results are shown in figure 4.

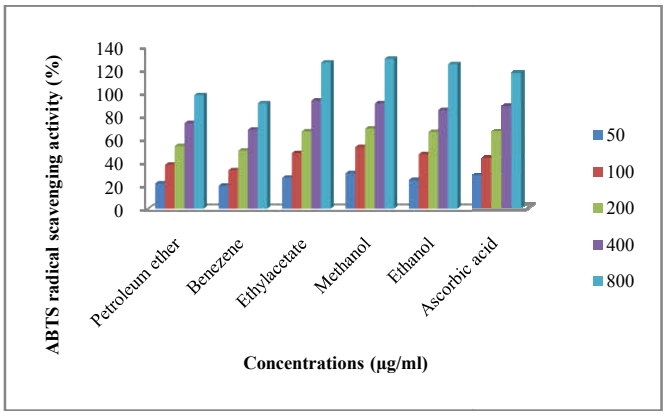

Figure 4 ABTS radical scavenging activity of various leaf extracts of *C.magna*

The methanol extract of *C.magna* leaf displayed potent ABTS radical cation scavenging activity in concentration dependent manner. At 800µg/ml concentration, the methanol extract of *C.magna* leaf possessed 129.37% ABTS radical cation scavenging activity respectively. The quantity of *C.magna* methanol extract required to produce 50% inhibition of ABTS radical 26.37 mg/ml whereas 30.13 mg/ml (Table 1) needed for trolox. The ABTS radical cation decolorization assay can measure the relative antioxidant capacity to scavenge the radical ABTS as compared with standard (Ascorbic acid), and is potential tool for evaluating the antioxidant activity of hydrogen donating antioxidants (Mathew and Abraham, 2004). The blue and green ABTS radical cation was generated prior to adding antioxidant containing samples prevents interference, which stable absorbance was achieved by adding the crude (methanol and ethanol) extracts of *C.magna*.

Reducing Power

Reducing power of the fractions was measured using ferric to ferrous reducing action as determined spectrophotometrically from the formation of Perl's Prussian blue colour complex (Yildirim *et al*., 2001). Figure 5 showed the reducing abilities of different solvent extracts of *C.magna* leaf 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 leaf of *C.magna* exhibited higher reducing activity. This result specifies that the extracts may consist of polyphenolic compounds that typically prove great reducing power. This has been justified by methanol extract being the most reducing agent with highest phenolic content

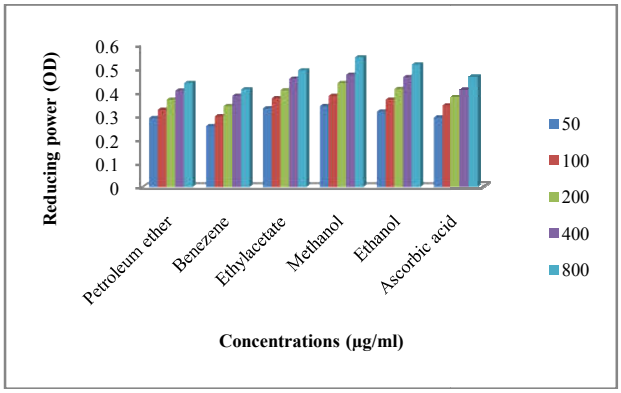

Figure 5 Reducing power ability of various leaf extracts of *C.magna*

The crude extracts of *C.magna* leaves performed varied levels of antioxidant activity in all the in-vitro models of antioxidant assays studied. The results from various free radical scavenging models exhibited that the *C.magna* had significant antioxidant activity and free radical scavenging activity. *C.magna* can be suggested as a potential natural source of antioxidants appropriate for utilization in nutritional or pharmaceutical fields.

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