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Antioxidant activity of leaves and bark extracts of *Crataeva magna* plant

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Abstract

Recently, a substantial body of literature provides strong evidence to support the potentials of *Crataeva magna* (CM), plant and its extracts in various pathological conditions. Thus, the CM plant extract at various concentrations were prepared and antioxidant activity was evaluated. The fresh leaves and bark of *CM* were collected and various extracts such as n-Hexane, Chloroform, Ethanol and aqueous extract were prepared. The preliminary phytochemical investigation showed the presence of various phytochemical such as total phenolic, flavonoid compounds and tannins. The ethanolic leaves extract of CM (ELECM) and water leaves extract of CM (WLECM), showed dose dependent increased in free radical scavenging activity in DPPH assay and reducing power assay in present study. Moreover, ethanolic barks extract of CM (EBECM) and water barks extract of CM (WBECM), also showed increased in free radical scavenging activity in DPPH assay and reducing power assay. However, the effects of bark extracts were lower than leaves extracts. In the light of the above consideration, the results of the study revealed that ELECM showed promising antioxidant activity in a dose dependent manner.

Keyword: Leaf Extract; Bark Extract; DPPH Assay; Reducing Power Assay; Antioxidant Activity

1. Introduction

Oxidative stress is a major supplier to the pathogenesis of a numeral of chronic diseases that is why antioxidant behavior is one of the most frequently determined biological activities in extracts of plants [1].Most of the organisms in this globe have well-organized resistance system to defend themselves against oxidative stress induced by ROS[2]. Studies reported that oxidative stress is defined as an imbalance between the production ROS and their cellular detoxification by antioxidants. Oxidative stress or free radicals are major contributor and involved in the pathogenesis of a number of chronic diseases such as myocardial infarction, Alzheimer's disease, Parkinson's disease, atherosclerosis, heart failure, and many more [3], that is why antioxidant performance is one of the most frequently determined biological behavior in various plants extracts [2]. On the other hand, several studies reported that antioxidants are mediators that scavenge the free radicals and avoid the damage caused by ROS and are vital for maintaining most favorable cellular activity, systemic health and well-being [4]. Generally there are two classes of antioxidants viz. non-enzymatic and enzymatic. The first class of non-enzymatic antioxidants such as flavonoids, ascorbic acid, and tannins are obtained from the plant species, whereas, enzymatic antioxidants produced endogenously and have glutathione, peroxidase (GSH), superoxide dismutase (SOD), and catalase (CAT).

Medicinal plants are now getting more attention than ever because they have potential of myriad benefits to society or indeed to all mankind especially in the line of medicine and pharmacology. Recently, a substantial body of literature

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provides strong evidence to support the Pharmacological potentials of *Crataeva magna (CM)*, plant and its extracts in various pathological conditions. CM is commonly known as Varuna. Varuna is one of the best litholytic herbs and has been used throughout the ages for the treatment of urolithiasis and crystalluria. Moreover, CM has been explored for its various medicinal properties viz. used in diseases like urinary disorders, urinary calculi, blood disorders, worms and tumors[5]. In this respect, different extract of this plant are expected to act as a potential strategy to scavenge free radicals. Thus, the CM (Capparidaceae), plant was selected for this study. Extract at various concentrations were prepared and antioxidant efficacy of extracts was evaluated.

2. Materials and methods

2.1. Plant Material

2.1.1. Collection and Authentication of CM Plant

Fresh leaves and bark of CM were collected from Tirumala hills, Chittoor district from the state of Andhra pradesh. The plant materials were taxonomically identified and authenticated by Dr. Madhava Chetty, Asst Professor, Dept. Of Botany, S.V. University, Tirupathy Andhra Pradesh, India and the sample voucher specimen and herbarium have been preserved in the Dept. Of Pharmacognosy, Luqman College of Pharmacy Gulbarga, Karnataka.

2.2. Preparation of leaf and bark extracts of CM

The leaves and stem bark of CM were collected. The leaves and bark of CM were washed thoroughly in water, dried for a week (35-400°C). Then leaves and stem bark were mechanically crushed and ground into powder. A portion (150 g) of the powdered of CM was weighed into a 1000 ml round bottom flask. The powder obtained was successively extracted with solvents of increasing polarity (hexane, chloroform, and ethanol) using Soxhlet apparatus. The resultant mixture was magnetically stirred in the round bottom flask for 72 hours and at room temperature and then allowed to stand for another 24 hours. The extracts were prepared by decanting, followed by filtration using cotton and Whatman filter paper to obtain a clear filtrate. The filtrate was evaporated to dryness at 50-60°C under reduced pressure in a vacuum rotary evaporator. Thus, crude extracts was obtained. Moreover, aqueous extract was also made by soaking 10 g of the weighed plant leaves and barks powder in 100 ml of boiled hot water. That mixture was boiled for thirty minutes into a conical flask and put for 24 h. The extract was filtered using filter paper and evaporated. The extract above obtained named as: n-Hexane extract, Chloroform extract, Ethanol extract and aqueous extract

2.3. Preliminary phytochemical screening of CM plant extracts [6,7]

All the extracts of three plants were subjected to preliminary phytochemical investigation for the detection of the following metabolites: (1) Alkaloids, (2) Carbohydrates, (3) Glycosides, (4) Phenolic compounds, (5) Flavonoids, (6) Protein and free amino acids, (7) Saponins (8) Sterols (9) Acidic compound, (10) Steroids, (11) Fixed oil and fats and (12) Terpenoids

2.4. Quantitative estimation of leaf and bark extracts of CM plants

2.4.1. Estimation of total phenolic content

According to the Folin–Ciocalteau method, the total phenolic (soluble) content was estimated using the Folin–Ciocalteau reagent. This method is based on the oxidation reaction. Gallic acid was used as standard reagent in this procedure. Extract solution (1.0 g/ml) was taken in the flask and then dilution of extract was made up to 46 ml with distilled water. After dilution, Folin–Ciocalteau reagent (1 ml) was added and mixed. After proper mixing, the solution was allowed to stand for 3 minutes. Further sodium carbonate was mixed into the above mixture solution and allowed to stand for 180 minutes by occasional shaking. Blue color developed was then noted at 760 nm. Phenolic contents in the extract were expressed in terms of mg of GAE /g of extract [8,9].

2.4.2. Estimation of tannin content

The tannin content in a sample was determined by Folin - Ciocalteu method. About 0.1 ml of the extract was taken in a (10 ml) volumetric flask containing 7.5 ml of distilled water, and 0.5 ml of Folin Phenol reagent, 1 ml of 35 % Na2CO3 solution and makeup the volume to 10 ml with distilled water. The mixture was well shaken and kept for 30 min in room temperature. A series (20, 40, 60, 80 and 100 µg/ml) of reference standard solutions of gallic acid was prepared in the same method as described earlier. Absorbance for test and standard solutions was measured against the blank at 725 nm in an UV/Visible spectrophotometer [10,11].

The tannin content and total phenol content was expressed in terms of mg of GAE/g of extract.

2.4.3. Determination of total flavonoid content

The aluminium chloride colorimetric method was employed to measure the total flavonoid content of a sample. In a 10 ml volumetric flask 1 ml of extract and 4 ml of distilled water along with 0.3 ml of 5 % sodium nitrite solution and 10 % aluminium chloride was added at an interval of 5 minutes. To this reaction mixture, 2 ml of 1M sodium hydroxide and 10 ml with distilled water was added after 5 minutes interval. A series (20, 40, 60, 80 and 100 µg/ml) of reference standard solutions of quercetin was prepared in the same method as described earlier. The absorbance for standard and test solutions was determined against the reagent blank at 510 nm in an UV/Visible spectrophotometer. The total flavonoid content was expressed in terms of mg of RE/g of extract [12,13].

2.5. *In-Vitro* antioxidant assay

2.5.1. Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity of leaf and bark extracts of CM plant

The in-vitro antioxidant activity of all plants extracts was measured by DPPH free radical scavenging assay method described earlier [14,15]. DPPH, a stable free radical has been extensively used to measure free radical scavenging activity of compounds. DPPH solution (0.1 mM) was prepared in ethanol and 1.0 ml of this solution was added to 3.0 ml of extract solution prepared in water at different concentrations (1-5 µg/ml). Mixture was incubated in dark for thirty minutes and the absorbance was measured at 517 nm. Ascorbic acid was used as the reference compound. The result of this method was expressed in the form of % DPPH scavenging effect and was calculated by the following formula:

Where A_0 was absorbance of blank and A_t absorbance in presence of extract. Test was carried out in triplicate.

2.5.2. Reducing power assay of leaf and bark extracts of CM plant for antioxidant activity

According to Oyaizu [16], reducing power was evaluated as follows, 2.5 ml volume of various concentrations of extracts (10-320 µg/ml) were mixed with sodium phosphate buffer (2.5 ml, 200 mM) and potassium ferricyanide (2.5 ml, 1%) at pH 6.6. Mixture was incubated (50 ˚C for 20 min). After incubation trichloroacetic acid (2.5 ml of 10% w/v) was added into the above solution. After proper stirring and mixing this solution was centrifuged (8 min at 1000 rpm) for separation of layers. After centrifugation of 8 min, upper layer was separated. This upper layer was taken for estimation. 5 ml of upper layer was added into deionized water (5 ml) and ferric chloride (1 ml, 0.1%). After proper mixing, absorbance was measured at 700 nm using double beam spectrophotometer. This procedure was repeated three times and means values ± S.D. were calculated. EC_{50} value was calculated from concentration-absorbance graph and ascorbic acid was used as standard.

2.6. Statistical Analysis

Data were presented as mean± S.EM. For continuous variables, student t-test was used to differentiate mean difference. For comparison between more than 2 group, the data were processed by one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. *$P < 0.05$ was considered significant. Statistical analysis was performed using SPSS version 21.

3. Results

3.1. Preliminary Phytochemical screening of the leaves and barks extracts of CM Plant

Phytochemical screening of the crude, 1:10 and 1:100 extracts of all the four solvents was performed in order to characterize the classes of compounds which are present in the leaves and barks (table 1). This qualitative screening included the tests for alkaloids flavonoids, tannins, phenolic, proteins and amino acids, carbohydrates, steroids, Saponins, cardio glycosides, terpenoids, fats and oils were performed by using reference methods.

The preliminary phytochemical analysis of leaf and bark of CM in this study revealed the presence of tannins, phenolic compounds, saponins, terpenoids, and flavonoids (table 1). This study revealed the presence of saponins in all solvent extracts of leaf sample of CM, whereas terpenoids were found in all the solvents leaf extracts. Besides, alkaloids were

present only in hexane extracts of leaf sample. While steroids, reducing sugar, carbohydrates and fixed oil and fat were totally absence in all the extracts. Moreover, ELECM, WELCM, EBECM and WEBCM found more rich in these phytoconstituents as compare to hexane and chloroform extracts (Table 1).

Table 1 Preliminary phytochemicals constituents in the leaf and bark extracts of CM plant

Phytochemicals	**Plant extracts**							
	Hexane		**Chloroform**		**Ethanol**		**Water**	
	Stem bark	Leaf	Stem bark	Leaf	Stem bark	Leaf	Stem bark	Leaf
Tannins	+	+	-	-	+	+	+	++
Phenols	++	+	+	+	++	++	+	++
Saponins	+	+	+	+	++	++	++	++
Terpenoids	-	+	-	+	-	+	-	+
Flavonoids	+	+	+	+	+++	+++	++	++
Steroids	-	-	-	-	-	-	-	-
Alkaloids	-	+	-	-	-	-	-	-
Glycosides/ reducing sugar	-	-	-	-	-	-	-	-
Carbohydrates	-	-	-	-	-	-	-	-
Fixed oils & fat	-	-	-	-	-	-	-	-

+-Indicate positive test results; -Indicate negative test results

3.2. Quantitative estimation of total phenolic, flavonoids and tannin content in the leaf and bark extracts of CM plant

The results for the total phenol, tannin and flavonoid estimation of all four extracts of **CM** are tabulated in **Table 2.**The total phenolic, tannin and flavonoids content of n-hexane, Chloroform, ethanol and aqueous extract s in leaf part was ranged from 0.8 -23.1, 0.0-2.5 and 1.4-38.4 g GAE/100 g extract respectively.

Table 2 Quantitative estimation of Phytoconstituents in leaf and bark extract of CM plant

Sample	**Total Phenolic mgGAE/g of extract**	**Total Tannins mgGAE/g of extract**	**Total Flavonoids mgRE/g of extract**
Leaf			
n-hexane	0.8 ± 0.1	Not Detected	1.4 ± 0.8
Chloroform	2.3 ± 0.9	0.9 ± 0.1	3.1 ± 1.4
Ethanol	23.1 ± 0.7	2.6 ± 0.1	38.4 ± 2.9
Water	19.1 ± 0.4	2.5 ± 0.5	17.6 ± 2.2
Bark			
n-hexane	1.4 ± 0.3	Not Detected	3.4 ± 0.7
Chloroform	4.7 ± 0.4	0.3 ± 0.0	10.5 ± 1.6
Ethanol	6.5 ± 0.9	1.9 ± 0.0	22.4 ± 1.3
Water	2.2 ± 0.4	1.0 ± 0.1	20.7 ± 0.6

Values are mean of 3 replicate determinations + SD. GAE- Gallic acid equivalent, RE- rutin equivalent

Whereas, the total phenolic, tannin and flavonoids content of n-hexane, chloroform, ethanol and aqueous extracts in bark part was ranged from 1.4 -6.5, 0.3-1.9 and 3.4-22.4 g GAE/100 g extract respectively (table 2). In the leaf and bark, ethanol extracts demonstrating highest total phenolic, tannin and flavonoid contents followed by water, chloroform and n-hexane extracts.

3.3. *In-vitro* Antioxidant activity

3.3.1. Determination of DPPH radical-scavenging activity of ELECM, WLECM, EBECM and WBECM

The extracts of CM exhibited significant ($p<0.05$) antioxidant activity assayed by DPPH scavenging activity (table 3). The antioxidant activity of FD leaves and bark extracts shown in Table 3. Ascorbic acid was used as a reference compound. All the estimations were done in triplicates and free radical scavenging activity was expressed in terms of IC_{50}. $IC_{50,}$ which is the concentration of the sample required to scavenge 50% of free radicals was calculated.

Ascorbic acid had shown IC50 value of 33.50 ± 0.74 µg/ml, whereas the IC50 of EBECM at a dose range of 1-600µg/ml was 100.45 ± 17.14, 87.67 ± 12.10, 56.34 ± 18.78, respectively. Whereas, the IC50 of ELECM at a dose range of 1-600µg/ml was 84.16 ± 10.72, 55.76 ± 16.98, 49.32 ± 9.14, respectively. IC50 of WBECM at a doses range of 1-600 µg/mlwas 135.43 ± 10.12, 128.76 ± 10.17, 104.97 ± 11.34, respectively whereas the IC50 of WLECM was 112.27 ± 17.23, 104.61 ± 7.39, 94.17 ± 12.30at doses of 1-600 µg/ml, respectively

The treatment with extracts produced dose dependent antioxidant activity and indicates the less antioxidant effect of the leaf was lower than the stem bark. But these values are found to be lower than the standard ascorbic acid.

Table 3 DPPH radical-scavenging activity of ELECM, WLECM, EBECM and WBECM

Samples	Treatment	Dose Concentration	IC50 Values
1	Ascorbic Acid	(1-10µg/ml)	33.50 ± 0.74 µg/ml
2	WLECM	(1-200µg/ml	112.27 ± 17.23µg/ml
3	WLECM	(1-400µg/ml)	104.61 ± 7.39µg/ml
4	WLECM	(1-600µg/ml)	94.17 ± 12.30µg/ml
5	ELECM	(1-200µg/ml	84.16 ± 10.72µg/ml
6	ELECM	(1-400µg/ml)	55.76 ± 16.98µg/ml
7	ELECM	(1-600µg/ml)	49.32 ± 9.14µg/ml
8	WBECM	(1-200µg/ml	135.43 ± 10.12µg/ml
9	WBECM	(1-400µg/ml)	128.76 ± 10.17µg/ml
10	WBECM	(1-600µg/ml)	104.97 ± 11.34µg/ml
11	EBECM	(1-200µg/ml	100.45 ± 17.14µg/ml
12	EBECM	(1-400µg/ml)	87.67 ± 12.10µg/ml
13	EBECM	(1-600µg/ml)	56.34 ± 18.78µg/ml

DPPH radical-scavenging activity of different concentration extracts and ascorbic acid. Data are represented as mean ± SD of three replicates

3.3.2. Determination of reducing power activity of ELECM, WLECM, EBEFD and WBECM

The ability of reducing power was maximum in leaf extracts when compared to other extracts (Table 4). The reducing power was moderate in bark extracts and the values were comparable to that of quercetin and BHA standards. This may be due to the biologically active compounds in the extract which possess potent donating abilities.

Table 4 Percentage of Scavenging Inhibition of ELECM, WLECM, EBECM and WBECM in reducing power activity

	(µg/mL)	**EBECM**	**ELECM**	**WLECM**	**WBECM**	**Standard Ascorbic Acid**
01	20	18.29±3.14	28.05±4.32	22.34±3.27	21.29±5.32	20.94±1.92
02	40	29.30±2.81	34.78±3.65	38.60±5.29	30.33±2.61	39.18±1.26
03	80	49.41±3.62	61.23±4.52	67.33±5.48	43.68±5.22	44.04±1.08
04	160	62.44±5.47	90.77±5.21	79.42±5.99	61.33±2.21	70.56±1.45
05	320	71.34±3.27	93.64±3.37	92.05±3.13	70.44±6.37	96.93±1.21

Reducing power activity of different concentration extracts and ascorbic acid. Data are represented as mean ± SD of three replicates.

4. Discussion

Medicinal plantsare moving from fringe to main stream use with a greater number of people seeking remedies and health approach. The medicinal plants are rich in secondary metabolites which include Alkaloids, Glycosides, Flavonoids, Steroids, Saponins, Phenols, Tannins and Terpenoids, which are of great medicinal value and have been extensively used in the drug and pharmaceutical industry. In this study the preliminary phytochemical analysis was carried out using various chemical reagents to find the nature and presence of the phytoconstituents in the extracts of the leaves and bark of CM plant.

The leaf and bark of CM show the presence of Tannins, Phenolic compounds, Saponines, Terpenoids, and Flavonoids. Moreover, the presence of Saponins and Terpenoids were found in all solvent extracts of leaf sample of CM. Besides, alkaloids were present only in hexane extracts of leaf sample. While steroids, reducing sugar, carbohydrates and fixed oil and fat were totally absence in all the extracts. Moreover, ELECM, WELCM, EBECM and WEBCM found more rich in these phytoconstituents as compare to hexane and chloroform extracts.

Further, quantitative estimation of total phenolic, tannins, and flavonoids compounds were also carried in the extracts. Out of all four extracts, the ELECM was rich in the phytoconstituents and showed higher amount of total Phenolic, Tannins, and Flavonoids compounds.

There has been increasing interest in natural antioxidants found in medicinal plants because of the carcinogenic effects of synthetic antioxidants [17]. Medicinal plants are good foundations with antioxidant property and are used as a substitute medicine to alleviate the diseases associated with oxidative stress [18,19]. The various parts of medicinal plants those are generally rich in various phytoconstituents such as tannins, Phenolic compounds, and flavonoids, have numerous biological effects as well as antioxidant activity.

In this study, the potential in-vitro antioxidant activity of plant extracts were estimated by DPPH radical scavenging and reducing power activity method. The ELECM and WBECM showed dose dependent increased in scavenging activity on free radicals in this study. The results indicate that both the extracts showed potential free radical scavenging activity in DPPH assay and ELECM has more DPPH radical scavenging activity than WBECM. In addition the ELECM and WBECM also showed dose dependent antioxidant activity in reducing power assays.

The data obtained in this study is supported by previous studies, those reported the anti-oxidant activity of leave and bark extract of CM. Previous studies reported that flavonoid, alkaloids and other constituents present in plant extract prevent oxidant injury by several mechanisms, such as scavenging oxygen radicals [20,21]. Several reports have conclusively shown close relationship between total Phenolic content and antioxidative activity of the plant extracts. Hence, the ELECM and WLECM could be a good source of antioxidant.

5. Conclusion

In this study the antioxidant activity of leave and bark extracts of CM plants was carried out. The result of this study concluded that with the support of the phytochemical studies, the in- vitro antioxidant activities were explored in a dose dependent manner.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest among all the authors.

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