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Original Research Article

Free Radical Scavenging and Antioxidant Activity of *Capparis Zeylanica* Linn

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Abstract

The aim of the present research was to evaluate the antioxidant potential of ethanol and aqueous extracts of *Capparis zeylania*. The free radical scavenging activity was found to be high in ethanol extract for DPPH, hydroxyl, superoxide, ABTS and reducing power assays in a concentration dependent manner followed by aqueous extract. The present results supported that the leaf extracts (ethanol and aqueous) of *C.zeylanica* could serve as potential alternative source antioxidants and can be explored as a good therapeutic agent in the free radical induced diseases.

Key words: Ascorbic acid, Trolox, Free radicals, ROS, Antioxidant.

INTRODUCTION

Medicinal herbs are an indispensable part of the traditional medicine practiced all over the world due to low costs, easy access and ancestral experience [1]. Now-a-days, vast numbers of people in developing and developed countries depend on plant based healthcare, skin therapy, commercial economic uses, and cultural development. The World Health Organization (WHO) reported that 80% of the people rely on traditional medicine, which is clearly elucidated by the 19.4 billion USD global revenue for herbal remedies in 2010 [2]. Therefore, the medicinal plants used in traditional medical treatments are significant in both developing and industrialized countries.

Capparis zeylanica Linn. (Caparadaceae) is commonly known as Indian caper, is a climbing shrub found throughout India and has been used as a 'Rasayana' drug in the traditional Ayurvedic system of medicine. It is a many branched thorny, sub-scandent climbing shrub. Plants are 2-3 m in height, armed with 3-6 mm long, leaves are elliptic or broadly lanceolate, base rounded, apex mucronate, flower profuse, pinkish white, later turning pink, berries are globular or elliposide, 3-4 cm in diameter, and seeds are globase, embedded in white pulp. The plant distributed throughout the major parts of India, Bangladesh and some parts of Pakistan. Leaves are used as folk medicine and as ingredient in various Ayurvedic preparations. Traditionally it is use as Antidote to snake bite, to cure swelling of testicle, small pox, boils, cholera, colic, hemiplagia, neuralgia, sores, pneumonic and pleurisy [3]. In North India, the leaves are widely used as counter- irritant, febrifuge and as a cataplasm in swellings and piles [4]. *Capparis* species has been reported to anthelmintic, antimicrobial activity [5] and anti-inflammatory activity [6]. In the present study, the crude extracts (ethanol and aqueous) of *C.zeylanica* were investigated for their antioxidant properties of DPPH, Hydroxyl, Superoxide, ABTS and reducing power assays.

MATERIALS AND METHODS

Plant collection

The fresh plant parts were collected from Kolli hills, Namakkal District, Tamil Nadu, and India. The collected plant was authenticated by Dr. S.P.Anand, Assistant Professor of Botany, PG & Research Department of Botany, National College, Tiruchirappalli, Tamilnadu, India and the voucher specimens were deposited at the herbarium of Department of Botany, National College (Autonomous), Tiruchirappalli-1.

Preparation of extracts

Plant material

Fresh and health leaves were collected from Kolli hills, Tamilnadu, India. The leaves were washed thoroughly in distilled water and the surface water was removed by air drying under shade. The leaves were powdered with the help of mechanical blender and used for extraction.

Preparation of extracts

Fifty gram of the coarse powder of *C.zeylanica* leaf was extracted successively with 100 ml of ethanol and aqueous by cold maceration methods. 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.

Chemicals

Ethanol, DPPH, Ascorbic acid and DMSO were of AR grade from Himedia (Mumbai, India), TCA, Ferric chlorite, Gallic acid, Quecetin, Potassium ferric cyanide, Sodium nitroprusside, Sulfanilamide, Naphthylethylenediamine dihydrochloride, Sodium carbonate, Hydrogen peroxide, Folin phenol reagent, Aluminum chloride and Potassium acetate were from SD Fine Chemicals (Mumbai, India).

DPPH assay

DPPH scavenging activity was carried out by the method of Blois [7]. Different concentrations of crude extracts were dissolved in DMSO (dimethyl sulfoxide) and taken in test tubes in triplicates. Then 5 ml of 0.1mM ethanol solution of DPPH (1, 1, Diphenyl-2-Picrylhydrazyl) was added to each of the test tubes and were shaken vigorously. They were then allowed to stand at 37^{o}C for 20 minutes. The control was prepared without any extracts. Methanol was used for base line corrections in absorbance (OD) of sample measured at 517nm. A radical scavenging activity was expressed as 1% scavenging activity and was calculated by the formula:

Percentage of radical scavenging activity = Control OD – Sample OD/ Control OD

Hydroxyl radical scavenging activity

As per the modified method of Halliwell *et al.* [8] the scavenging ability for hydroxyl radical was estimated. Using dilute deionized water, Stock solutions of EDTA (1 mM), $FeCl_3$ (10 mM), H_2O_2 (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 $FeCl_3$, 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^{o}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 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 anion radical scavenging

The superoxide anion scavenging activity was computed as depicted by Srinivasan *et al.* [9]. 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^{o}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 = (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.

ABTS Radical Cation

ABTS assay was based on the slightly modified method of Huang *et al.* [10]. 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 assay

Reducing activity was carried out by using the method of Oyaizu [11]. Different concentrations of crude extracts were prepared with DMSO and taken in test tube as triplicates. To test tubes 2.5 ml of sodium phosphate buffer and 2.5 ml of 1% Potassium ferric cyanide solution was added. These contents were mixed well and were incubated at 50^{o}C for 20 minutes. After incubation 2.5ml of 10% Trichloroacetic acid (TCA)

was added and were kept for centrifugation at 3000rpm for 10 minutes. After centrifugation 5ml of supernatant were taken and to this 5ml of distilled water was added. To this about 1ml of 1% ferric chlorite was added and was incubated at 35°C for 20 minutes. The OD (absorbance) was taken at 700nm and the blank was prepared by adding every other solution but without extract and ferric chloride (0.1%) and the control was prepared by adding every other solution but without extract. The reducing power of the extract is linearly proportional to the concentration of the sample.

RESULTS AND DISCUSSION

Reactive oxygen species (ROS) including superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide are often generated as byproducts of biological reaction or from exogenous factor. Plants are rich in antioxidants; so much attention has been directed towards the development of ethnomedicines as they contain phenols, flavonoids, alkaloids, tannins, vitamins, terpenoids, and many more phytochemicals responsible for different pharmacological activities. The antioxidant activity of *C.zeylanica* have been evaluated by measuring their DPPH, Hydroxyl, Superoxide, ABTS, reducing power ability and ascorbic acid/Trolox content using crude extracts of leaves. The 1,1-diphenyl-2-picryl hydroxyl (DPPH) radical was repeatedly used as the model system to evaluate the ability of antioxidant properties of several natural compounds such as extracts of plants in a relatively short time. The method is based on the reduction of methanol DPPH solution in the presence of a hydrogen donating antioxidant due to formation of the non-radical form DPPH –H by the reaction. The extract was able to reduce the stable radical DPPH to the yellow colored diphenyl picrylhydrazine. It has been found that cysteine, glutathione, ascorbic acid, tocopherol, poly-hydroxy aromatic compounds reduce and decolorize DPPH by their hydrogen donating ability [7]. DPPH free radical scavenging properties of ethanol and aqueous extracts of leaf of *C.zeylanica* are shown in table 1. The scavenging effect of standard (ascorbic acid), and crude extracts exhibited increases with the increase in the concentration. Among the two extracts studied, ethanol extract of *C.zeylanica* reveled the highest DPPH radical scavenging activity.

Table-1: Effect of Ethanol and Aqueous extracts of *C.zeylanica* on DPPH assay

Concentration (µg/ml)	Antioxidant activity (%)		
	Ethanol	Aqueous	Ascorbic acid
50	32.33 ± 0.57	24.03 ± 0.25	25.81 ± 0.10
100	41.0 ± 1.0	31.23 ± 0.49	35.08 ± 0.46
200	60.46 ± 0.80	45.73 ± 0.30	58.51 ± 0.13
400	70.16 ± 0.28	59.33 ± 0.25	66.43 ± 0.24
800	81.9 ± 0.85	75.71 ± 0.41	79.23 ± 0.43
IC_{50}	38.01 ± 0.18	40.2 ± 0.24	36.7 ± 0.28

Each value is expressed as percentage of activity mean ± standard deviation (n=3)

The hydroxyl radical scavenging activity of the ethanol and aqueous extracts was investigated (Table 2). The crude extracts exhibited strong concentration-dependent scavenging abilities for the hydroxyl radical. Ethanol extract was found to be the most powerful scavenger of the hydroxyl radical, with an inhibition of up to 80.7 % at a concentration of 800µg/ml. The moderate scavenger was found to be aqueous, the inhibition of which, however, reached 63.8 % at 800µg/ml. The results showed that the extracts obtained by cold-extraction had excellent scavenging activities for the hydroxyl radical. Hydroxyl radicals are highly strong reactive oxygen species, and there is no specific enzyme to defense against them in human body [12]. Therefore, it is important to discover some chemicals with good scavenging capacity on these reactive oxygen species. In this study, the hydroxyl radical scavenging ability of ethanolic and aqueous extracts were compared with ascorbic acid showed more pronounced hydroxyl radical scavenging activity in a dose dependent manner.

Table-2: Effect of Ethanol and Aqueous extracts of *C.zeylanica* on Hydroxyl assay

Concentration (µg/ml)	Ethanol	Aqueous	Ascorbic acid
50	25.83 ±0.37	19.16 ± 0.30	21.43 ± 0.14
100	45.4 ± 0.45	27.56 ± 0.30	38.54 ± 0.23
200	68.96 ± 0.57	38.2 ± 0.26	45.09 ± 0.76
400	75.43 ± 0.37	55.66 ± 0.35	73.70 ± 0.29
800	80.7 ± 0.36	63.8 ± 0.26	79.61 ± 0.48
IC_{50}	32.8 ± 0.45	39.1 ± 0.54	29.6 ± 0.21

Each value is expressed as percentage of activity mean ± standard deviation (n=3)

Superoxide anions convert to oxygen and hydrogen peroxide by superoxide dismutase, or they react with nitric oxide to form peroxynitrite. Hydrogen peroxide can be converted into water and oxygen by catalase. Therefore, superoxide scavenging capacity in the human body is very important as the first line of

protection against oxidative stress [13]. The superoxide anion radical scavenging activities (%) of crude extracts (ethanol and aqueous) *C.zeylanica* were significantly equal to standard. Superoxide radical scavenging capacities of the crude extracts tested varied from 18.6 to 74.3 % which represents a variation of standard ascorbic acid. Ethanol extract showed the highest antioxidant capacity (74.3%) followed by aqueous (65.53%) (Table 3).

Table-3: Effect of Ethanol and Aqueous extracts of *C.zeylanica* on Superoxide anion radical scavenging assay

Concentration (µg/ml)	Ethanol	Aqueous	Ascorbic acid
50	27.06 ± 0.40	18. 46 ± 0.25	27.43 ± 0.34
100	44.6 ± 0.49	25.56 ± 0.15	43.41 ± 0.28
200	56.4 ± 0.00	46.7 ± 1.91	56.13 ± 0.21
400	64.53 ± 0.25	56.6 ± 0.26	66.54 ± 0.29
800	74.3 ± 0.3	65.53 ± 0.15	79.39 ± 1.02
IC_{50}	36.5 ± 0.27	38.6 ± 0.23	31.8 ± 0.65

Each value is expressed as percentage of activity mean ± standard deviation (n=3)

The scavenging potential of the crude extracts (ethanol and aqueous) for the ABTS radical were analyzed and compared (Table 4). As can be seen, the scavenging effect of the crude extracts increased with increasing concentration. As in the case of DPPH radical scavenging, ethanol exhibited the highest ABTS antiradical properties, followed by aqueous with an inhibition of 68.0 % for the ABTS radical at 800µg/ml. The order of ABTS radical scavenging activity of all extracts was similar to that observed for DPPH. The differences in the ABTS scavenging activities exhibited by the various extracts indicated that the extracting solvent and extraction method influenced the antioxidant ability of the extracts. The ABTS radical action is reactive towards most antioxidants including phenolics, thiols and Vitamin C. During this reaction, the blue ABTS radical cation is converted back to its colorless neutral form. The reaction may be monitored spectrophotometrically. The scavenging activity of ABTS+ radical by the plant extract was found to be appreciable; this implies that the plant extract may be useful for treating radical related pathological damage especially at higher concentration [14].

Table-4: Effect of Ethanol and Aqueous extracts of *C.zeylanica* on ABTS assay

Concentration (µg/ml)	Ethanol	Aqueous	Trolox
50	29.83 ± 0.25	18.43 ± 0.32	28.42 ± 0.27
100	41.63 ± 0.32	31.36 ± 0.25	42.54 ± 0.11
200	56.56 ± 0.35	50.33 ± 0.23	59.51 ± 0.27
400	65.5 ± 0.4	61.33 ± 0.577	66.32 ± 0.93
800	71.8 ± 0.3	68.0 ± 0.7	70.45 ± 0.21
IC_{50}	34.78 ± 0.45	35.8 ± 0.04	32.7 ± 0.71

Each value is expressed as percentage of activity mean ± standard deviation (n=3)

Reducing power assay is often used to evaluate the ability of natural antioxidants to donate electron or hydrogen [15]. Samples with high reducing power were reported to have a better ability to donate electrons. It has been widely accepted that the higher level of absorbance at 700 nm indicates greater reducing power of the test sample [16]. In this assay, samples were assayed in redox-linked reaction, whereby the antioxidants present in the samples act as the reductants, while the reagent containing excess of ferric ions act as oxidants. Reduction of the ferric chloride into ferrous complex can be measured at 700 nm. The intensity of the colour is related to the amount of antioxidant reductants present in the sample. Many research efforts have been revealed that there was a direct correlation between antioxidant activity and reducing power [16]. Table 5 indicates the reductive capabilities of the ethanol and aqueous extracts of *C.zeylanica*. In the concentration range investigated, the crude extracts showed reducing power that increased linearly with concentration. At 50, 100, 200, 400 & 800 mg/ml, reducing power of ethanol extract of *C.zeylanica* were found to be 0.183, 0.290, 0.418, 0.594 & 0.721. Among the two extracts tested, the ethanol extract exhibited significant reducing capacities.

Table-5: Effect of Ethanol and Aqueous extracts of *C.zeylanica* on reducing power

Concentration (µg/ml)	Ethanol	Aqueous	Ascorbic acid
50	0.183 ± 0.002	0.098 ± 0.003	0.245 ± 0.02
100	0.290 ± 0.004	0.159 ± 0.001	0.324 ± 0.31
200	0.418 ± 0.03	0.269 ± 0.002	0.487 ± 0.87
400	0.594 ± 0.02	0.412 ± 0.002	0.671 ± 0.03
800	0.721 ± 0.03	0.543 ± 0.003	0.765 ± 0.05

Each value is expressed as percentage of activity mean ± standard deviation (n=3)

CONCLUSION

In the present study, the ethanol and aqueous extracts of *C.zeylanica* exhibited significant antioxidant activity. Among the two extracts used in the antioxidant activity, ethanol extracts of *C.zeylanica* showed maximum and good activity. *C.zeylanica* is thought to be used as additives for food products and pharmaceutical industries with appropriate antioxidant properties and an antioxidant in future studies of experimental animal models, against free radicals generated in response to oxidative stress; also, it is thought that these data will be reference for future studies.

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