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Research Article

ANTIOXIDANT POTENTIAL OF DRIED ROOT POWDER OF *CAPPARIS ZEYLANICA* LINN

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ABSTRACT

The present study was aimed to investigate the antioxidant activity of extracts of dried root powder of *Capparis zeylanica* Linn. (Family: Capparidaceae). *Capparis zeylanica* Linn. has been reported to possess anthelmintic, antimicrobial and immunostimulant activity. Modern phytochemical screening of the plant has shown the presence of fatty acids, flavonoids, tannins and alkaloids. In evaluation, ethanol and methanol extracts were prepared and screened for in-vitro antioxidant activities by 1,1-diphenyl-2- picrylhydrazyl (DPPH) free radical scavenging activity and by reducing power assay method. The results of both the methods were compared with a natural antioxidant ascorbic acid (vitamin C) as a standard. The percentage scavenging of hydrogen peroxide at 60 µg/ml of methanolic extract, ethanolic extract and standard were 50.07%, 58.81% and 90.11% respectively. The IC50 value of methanolic extract, ethanolic extract and standard ascorbic acid were found to be 57.23 µg/ml, 43.98 µg/ml and 9.68 µg/ml respectively. Both the extracts showed strong antioxidant activity in these methods. Amongst these two extracts, ethanolic extract has shown better antioxidant activity as compared to methanolic extract.

Keywords: Antioxidant, *Capparis zeylanica*, free radical scavenging activity, reducing power.

INTRODUCTION

Reactive oxygen species (ROS), which include free radicals such as superoxide anion radicals ($O_2^{\cdot-}$), hydroxyl radicals ($OH^{\cdot}$), non-free-radical species such as H_2O_2 and singlet oxygen ($^1O_2$) are various forms of activated oxygen. The importance of free radicals and ROS has attracted increasing attention over the past decade.[1]

Objective

Recently interest has increased considerably in finding natural occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants that are being restricted due to their side effects such as carcinogenicity. One among such natural plants is *Capparis zeylanica* Linn. Commonly known as Indian caper, a climbing shrub found throughout India. The leaves are widely used as counter- irritant, febrifuge and as a cataplasm in swellings and piles.

Capparis zeylanica has been reported to possess anthalmintic and antimicrobial, and immunostimulant activity. Modern phytochemical screening of the plant has shown the presence of fatty acids, flavonoids and alkaloids.[2-5]

MATERIALS AND METHODS

Chemical and Instrument

Chemicals: ascorbic acid, hydrogen peroxide, potassium ferricyanide, trichloroacetic acid, ferric chloride, all other reagents used were of analytical grade.

Instrument: UV spectrophotometer (Shimadzu- UV-1601), centrifuge machine (Eltekresearch centrifuge-TC-4100D).

Preparation of plant extract

The dried root powder (150 gm) of *Capparis zeylanica* Linn. was extracted successively with 750 ml each of ethanol and methanol in a soxhlet extractor for 6 hrs. The solvent was removed by distillation and the semisolid mass was dried using hot water bath at 40-50ºC. The ethanol extract yield a dark brownish solid residue weighing 2.601 gm (1.73% w/w) and methanol extract yield a dark brownish solid residue weighing 1.023 gm (0.682% w/w) respectively.

Preparation of *capparis zeylanica* stock solution

Methanolic and Ethanolic extracts of *Capparis zeylanica* were prepared at the concentration of 1000 µg/ml in methanol. From the stock solution, different concentration viz. 10, 20, 30, 40, 50, 60, 80, 100 and 200 µg/ml were prepared in methanol and used for antioxidant studies.

Preparation of standard stock solution

Ascorbic acid used as standard for the study and its stock solution was prepared in the concentration of 1000 µg/ml in methanol. It was prepared freshly and used immediately for the study. From the stock solution, different concentration viz.10, 20, 30, 40, 50, 60, 80, 100 & 200 µg/ml were prepared in methanol and used for antioxidant studies.

Determination of total antioxidant activity

Reducing power assay

The reducing power of ethanolic extract of dried root powder of *Capparis zeylanica* Linn. was determined by the method of Oyaizu 1986[6]. According to this method various concentrations of the extracts (1 to 16µg/ml) in 1.0 ml of deionized water were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). The mixture was incubated at 50^0 C for 20 min. Aliquots of trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5ml). The absorbance was measured at 700 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations (1 to 16µg/ml) was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power.

$$\% \text{ Increase in reducing power} = [(A_{test}/A_{control}) -- 1] \times 100$$

A_{test} is absorbance of test solution

$A_{control}$ is absorbance of blank.

The antioxidant activity of the wood extract was expressed as EC50 and compared with standard.

DPPH free radical scavenging activity

The free radical scavenging activity was followed by the DPPH method[7]. 0.1mM solution of DPPH in methanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in methanol at different concentration (1-100µg/ml). Thirty minutes later, the absorbance was measured at 517 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations (1 to100 µg/ml) was used as standard. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation.

$$\text{DPPH Scavenged (\%)} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Where $A_{control}$ is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the wood extract was expressed as IC50 and compared with standard. The IC50 value was defined as the concentration (in µg/ml) of extracts that scavenges the DPPH radicals by 50%.

RESULTS AND DISCUSSION

Reducing power assay

Reducing power assay method is based on the principle that substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. The reducing power of the methanolic and ethanolic extracts increases with the increase in amount of sample (Table 1).

DPPH Free radical scavenging activity

In free radical scavenging activity, DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become stable diamagnetic molecule. The reduction capability of DPPH radical was determined by the decrease in its absorbance at 517 nm, which is induced by different antioxidants (Table 2).

Table 1: Shows the Absorbance of Standard, methanol extract and ethanol extract at various concentrations (µg/ml) in ferric reducing power determination model

Concentration (µg/ml)	Absorbance		
	Standard*	Methanol*	Ethanol*
10	0.070±0.002	0.050±0.002	0.045±0.002
20	0.081±0.001	0.079±0.002	0.057±0.001
40	0.110±0.002	0.086±0.003	0.063±0.002
60	0.152±0.002	0.093±0.001	0.134±0.002
80	0.195±0.003	0.096±0.002	0.164±0.003
100	0.226±0.001	0.110±0.001	0.167±0.004
200	0.321±0.004	0.138±0.002	0.193±0.001

*Each Value represents Mean ± SEM (n=3)

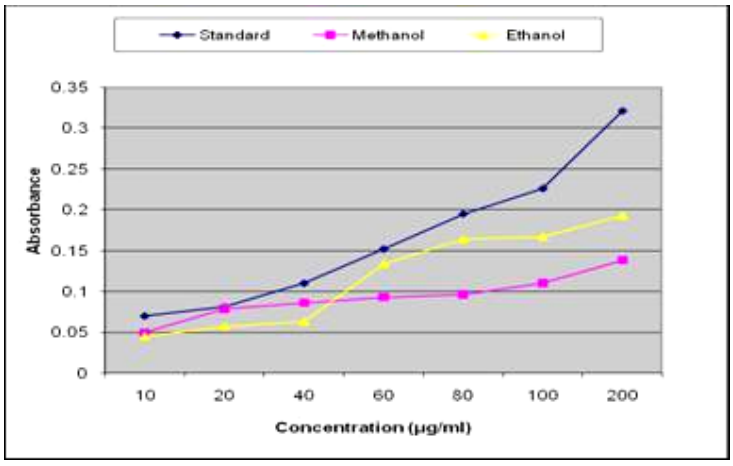

Fig. 1: Ferric reducing power determination of methanolic and ethanolic extract of *Capparis zeylanica* Linn. and standard ascorbic acid.

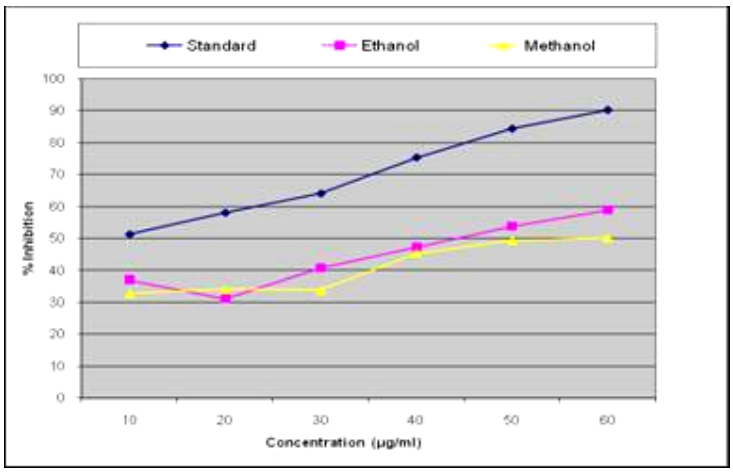

Fig. 2: Hydrogen peroxide scavenging activity of ethanol and methanol extracts of *Capparis zeylanica* and standard ascorbic acid

Table 2: Shows percentage inhibition of standard, ethanol and methanol extracts at various concentrations (µg/ml) in hydrogen peroxide scavenging model

Concentration (µg/ml)	% Inhibition		
	Standard*	Methanol*	Ethanol*
10	51.34±0.37	32.71±0.33	37.01±0.33
20	58.08±0.27	34.05±0.50	31.14±0.74
30	64.07±0.32	33.83±0.87	40.73±0.55
40	75.30±0.24	45.35±0.32	47.21±0.44
50	84.28±0.52	49.25±0.24	53.86±0.50
60	90.11±0.74	50.07±0.74	58.81±0.32

*Each Value represents Mean ± SEM (n=3)

CONCLUSION

It is well known that free radicals are one of the causes of several diseases. The result from the two in-vitro antioxidant model reveals that the root powder extracts of *Capparis zeylanica* Linn. had significant antioxidant activity. The exact constituents that show free radical scavenging action are unclea.

However, the observed antioxidant activity of the extract of *Capparis zeylanica* Linn. may be due to the presence of tannins found in preliminary phytochemical investigation.

Thus, to conclude *Capparis zeylanica* Linn. root powder extract showed antioxidant activities hence further studies are needed to evaluate the *in-vivo* antioxidant potential of these extracts in various animal models.

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