EVALUATION OF ANTIOXIDANT ACTIVITIES IN ETHANOLIC EXTRACT OF *CAPPARIS ZEYLANICA* LINN. ROOT.

Sunil Kumar Mishra*[a], P. N. Singh[a], S. D. Dubey[b]

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ABSTRACT

The *in vitro* antioxidant activity of the *Capparis Zeylanica* extract showed that has a scavenging ability of hydroxyl peroxide radicals (421.74 ± 25.61 mg/ml) and DPPH radical scavenging activity (around 95%). *In vivo* study in rats with *Capparis Zeylanica* extract showed significant antioxidant activity in the DPPH test as compared to the control. *Capparis Zeylanica* extract at doses of 100 and 200 mg/kg significantly increased rats TAP as compared to control where as the decreased in TBARS was significant in 200 and 400 mg/kg compared to the control. Administration of *Capparis Zeylanica* extracts at a dose of 400 mg/kg per day did not significantly alter serum TAP but little alteration was observed in TBARS test. Antioxidant activities of the extract at doses of 100 and 200 mg/kg were in all experiments comparable to that of α–tocopherol (10 mg/kg). A good result for DPPH and TBARS was found at a dose of 400 mg/kg compared to the α–Tochopherol. Dose dependent effect was found in DPPH scavenging activity. The constituents like total phenolic compounds (43.625±1.45 mg/g of dry extract), total tannins (24.75±1.23 mg/g of dry extract), total flavonols (1.653 ± 1.41mg rutin equivalents/g dry extract), total flavonoids (0.876±0.26 mg/g Rutin/g of dry extract), Saponins (1.22±1.35 mg/g of dry extract) and fatty acids (0.0867 ± 0.01 mg/g of dry extract) indicate that the *Capparis Zeylanica* root extract may contribute to the antioxidant activity. *www.relaquim.com*

Keywords: *Capparis Zeylanica*, DPPH, TAP, FRP, TBARS, phenolics.

RESUMEN

La actividad antioxidante *in vitro* de los extractos de *Capparis Zeylanica* mostró que tienen la habilidad atrapadora de radicales hidroxil peróxido (421.74 ± 25.61 mg/ml) y actividad atrapadora de radicales DPPH (alrededor del 95%). El estudio *in vivo* en ratas con extractos de *Capparis Zeylanica* mostró actividad antioxidante significativa en la prueba de DPPH comparado con el control. Los extractos de *Capparis Zeylanica* a las dosis de 100 y 200 mg/kg incrementaron significativamente el TAP en ratas comparados con el control, en tanto que la disminución en

[a]Indian Institute of Technology (BHU), Varanasi 221 005.
[b]Institute of Medical Science (BHU), Varanasi 221 005.
Skmishra.phe@itbhu.ac.in Ph. No.- 09793523844, 05426702093

el ensayo TBARS fue significativo a 200 y 400 mg/kg comparado con el control. La administración de extractos de *Capparis Zeylanica* a la dosis de 400 mg/kg por día no alteró el TAP en suero y se observaron pocas alteraciones en el ensayo de TBARS. La actividad antioxidante de los extractos a las dosis de 100 y 200 mg/kg en todos los experimentos fueron comparables al de α–tocoferol (10 mg/kg). Se encontró un buen resultado para DPPH y TBARS a la dosis de 400 mg/kg comparable al de α–tocoferol. Se encontró un efecto dosis-dependiente en la actividad atrapadora del radical libres en la prueba DPPH. Los constituyentes como compuestos fenólicos totales (43.625±1.45 mg/g de extracto seco), taninos totales (24.75±1.23 mg/g de extracto seco), flavonoles totales (1.653 ± 1.41mg equivalentes de rutina/g extracto seco) y ácidos grasos (0.0867 ± 0.01 mg/g de extracto seco) indica que el extracto de raíz de *Capparis Zeylanica* puede contribuir a la actividad antioxidante. *www.relaquim.com*

INTRODUCTION

Antioxidants have the ability of protecting organisms from damage caused by free radical-induced oxidative stress (Li L.J., 1999). Presently, the probable toxicity of synthetic antioxidants has been condemned. It is strongly believed that regular consumption of plant-derived phytochemicals may drift the balance toward an adequate antioxidant status (Halliwell, B, 1996). Thus, in recent years, interest on natural antioxidants, especially of plant origin, has increased manifolds (Jayaprakash *et al.*, 2000). *Capparis Zeylanica*, variously known as Indian caper, a climbing shrub found throughout India belonging to family Capparidaceae. In Sanskrit it is known as Vyakhranakhi, kinkani, tapasapriya, granthila, karambha (Satyanarayana *et al.*, 2008). It grows in moist habitat and is found throughout the majour parts of India. In different parts of India it is known with different names like Asadhua in Orissa, Kathotti in tamil etc (Muthu *et al.*, 2006). Almost all the parts ie. Root, bark, fruits, leaves, fruits, seeds are used for different purposes, it is used in many ayurvedic formulations (Mishra *et al.*, 2011). *Capparis Zeylanica* Linn. (Capparidaceae) has been used as a (Rasayan) drug in the Ayurvedic system of medicines. *Capparis Zeylanica* Linn. is reported to posses anti oxidant, sedative, antipyretic, analgesic, anti-inflammatory, antimicrobial and immunostimulant activity. This plant has been of interest to researchers because it is a medicinal plant employed in Indian traditional system of medicine. Root is astringent, diuretic, germicidal, and abortifacient. Root bark ten gram with one cup water crush and filter; this juice is narcotic, useful for relieving accidental pain (Varsha *et al., 2011*). It contains alkaloids, phytosterols, mucilaginous substances and water soluble acid. (Chakravarti *et al.*, 1932) The plant possesses sedative property (Schults *et al.*, 1984 and Jain *et al.* 1994). Despite its wide range of folk medicinal uses in India sub-continent, there is very little documentation on its phyto- constituents and antioxidant activity is available especially for the root part. A review of the literature throws a little light on the antioxidant study of this plant. The aim of this study was to assess the *in vitro* and *in vivo* antioxidant activity of the ethanolic extract of *Capparis Zeylanica* root. For this purpose, the factors responsible for the potent antioxidant have been evaluated for the total free radical scavenging activity, preliminary phytochemical assay, hydrogen peroxide ability and hydroxyl inhibitory activity. Attempts have also been made to quantitatively identify important phytochemicals (phenolics) and correlate these constituents with the free radical scavenging reactions.

MATERIALS AND METHODS

Chemicals and Reagents

2,2-diphenyl-1-picryl-hydrazyl (DPPH), Rutin, sodium nitrite ($NaNO_2$), trichloroacetic acid (TCA), ascorbic acid, ferric chloride ($FeCl_3$), 2-deoxy-2-ribose, butylated hydroxytoluene (BHT), gallic acid, magnesium ribbon, acetic anhydride and ammonium hydroxide (NH_4OH) were obtained from Hi Media Laboratories Pvt. Ltd, Mumbai, India. Thiobarbituric acid (TBA) was obtained from Loba Chemical, Mumbai, India. Ethanol, chloroform, ethanol, glacial acetic acid, benzene, hydrogen peroxide, ethylenediamine tetraacetic acid (EDTA), potassium di-hydrogen phosphate (KH_2PO_4), di-potassium hydrogen phosphate (K_2HPO_4), potassium hydroxide (KOH), sodium hydroxide (NaOH), potassium ferricyanide ($K_3Fe(CN)_6$), Fehling's solution, Mayer's reagents, sodium carbonate (Na_2CO_3), conc. HCl and conc. H_2SO_4 were procured from Merck, Mumbai, India. Folin-Ciocalteu reagent was from Sisco Research Laboratory, Mumbai, India. Aluminum chloride ($AlCl_3$) was obtained from SD Fine Chemicals Limited, Mumbai, India. All chemicals and solvents are analytical grade. All chemicals were of highest purity (99.0%). Sodium acetate, 2,4,6-tripyridyl-striazine (TPTZ), 1,1,3,3-tetramethoxypropan (MDA), trichloroacetic acid (TCA), glacial acetic acid, $FeCl_3{\cdot}6H_2O$, HCl, α–Tocopherol (Trolox) and *n*-butyl alcohol were purchased from Merck India.

Plant Material and Extraction

Roots of *Capparis Zeylanica* Linn. (*C. horrida* Linn., *Capparis brevispinia* DC.), were collected in the month of March from Medicinal Plant garden of Banaras Hindu University, varanasi campus. It was identified, confirmed and authenticated by comparison with an authentic specimen. A voucher specimen (H. P. L. 512) of the plant including the root is deposited in the department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi for future reference. Collected fresh root was washed, dried and 10g of root powder was extracted in a Soxhlet apparatus using 90% ethanol (the ratio of plant material to solvent was 1:15 m/v). The extraction was carried out at boiling temperature for 6 h. The extracts obtained were evaporated under pressure at 50 º C to a constant weight. The extract was stored at 4ºC until required. Before use, the *Capparis Zeylanica* root extract was dissolved in double-distilled water in desired concentrations.

Determination of Plant Extract Yield

The yield of evaporated dried *Capparis Zeylanica* root extract based on dry weight basis was calculated from the following equation:

$$\text{Yield (g/100 g of dry plant material)} = (W1 \times 100) / W2$$

Where, W1 and W2 were the weight of the extract after the solvent evaporation and the weight of the dry plant material, respectively.

Preliminary Phytochemical Screening

Tests for sterols/triterpenes, phenolic compounds, flavonoids, tannins, carbohydrates/ glycosides, saponins and alkaloids were carried out according to the previously reported method (Harborne JB, 1973). Phytochemical screening for the presence of secondary metabolites was performed using TLC analyses (precoated aluminium silica gel plates, GF254, Merck) with different eluting systems. The solvent systems were (CH_2Cl_2: MeOH, 18:1),$CHCl_3$:EtOAc:MeOH; 2:2:1), ($CHCl_3$: MeOH, 8:1 and 8:2), ($CHCl_3$:MeOH:H_2O; 62:35:5),(n-BuOH:MeOH:H_2O, 5:1:1). Spray reagents used in order to develop the spots were: 1% ferric chloride (tannins), 2% aluminium chloride in ethanol (flavonoids), 40% sulphuric acid/ethanol (saponins), and Dragendorff reagent (alkaloids) (Wagner *et al.*, 1984). Also, one dimensional PC was performed on Whatmann No. 1 (57 _ 46 cm) using BAW solvent systems (n-BuOH:

AcOH: H_2O, 4:1:5 organic layer) and 15% AcOH/H_2O. The change of spot colours on the chromatograms was detected by exposing to ammonia vapour or spraying with 1% ethanol $AlCl_3$ or $FeCl_3$ (Mabry & Thomas, 1970).

Estimation the total amount of phenolic compounds, flavonoids, flavonols, fatty acids and tannins

Determination of the Total Phenol Content

The total phenolic, flavonoid, flavonol and tannin contents of each active fraction was measured according to the methods previously described for total phenolics, flavonoids and flavonols (Kumaran & Karunakaran, 2006). The total phenolic content of plant extracts was determined using FCR. This method depends on the reduction of FCR by phenols to a mixture of blue oxides which have a maximal absorption in the region of 750 nm. About 100 µl of plant extracts (100 µg/ml) and also 100 µl of gallic acid (100 µg/ml) were mixed with 500 µl of the FCR and 1.5 ml of 20% sodium carbonate. The mixture was shaken thoroughly and made up to 10 ml using distiled water. The mixture was allowed to stand for 2 h. Then the absorbance at 765 nm was determined against a blank which contain all reagents without the samples or the gallic acid at the same conditions. All determinations were carried out in duplicates. The total phenolic content is expressed as the number of equivalents of gallic acid.

Determination of the Total Flavonoid Content

The flavonoids content was determined by aluminium chloride method using rutin as a reference compound. This method based on the formation of a complex flavonoid-aluminum having the absorptivity maximum at 415 nm. About 100 µl of plant extracts in ethanol (10 mg/ml) was mixed with 100 µl of 20% aluminium trichloride in ethanol and a drop of acetic acid, and then diluted with ethanol to 5 ml. The absorption at 415 nm was read after 40 min. Blank samples were prepared from 100 µl of plant extracts and a drop of acetic acid, and then diluted to 5 ml with ethanol. The absorption of standard rutin solution (0.5 mg/ml) in ethanol was measured under the same conditions. All determinations were carried out in triplicates. The amount of flavonoids in plant extracts in rutin equivalents was calculated by the following formula:

$$X = (A.\ m_o) / (A_o.\ m)$$

where X is the flavonoid content, mg/mg plant extract in rutine equivalent, A is the absorption of plant extract solution, Ao is the absorption of standard rutin solution, m is the weight of plant extract, mg and mo is the weight of rutin in the solution, mg.

Determination of the Total Flavonol Content

The content of flavonols was determined by using rutin as a reference compound. This method also based on the formation of complex with maximum absorption at 440. About 1 ml of each ethanolic plant extract (10 mg/ml) was mixed with 1 ml aluminium trichloride (20 mg/ ml) and 3 ml sodium acetate (50 mg/ ml). The absorbance at 440 nm was read after 2.5 h. The absorption of standard rutin solution (0.5 mg/ml) in ethanol was measured under the same conditions. All determinations were carried out in triplicates. The amount of flavonols in plant extracts in rutin equivalents (RE) was calculated by the same formula of flavonoids:

$$X = (A\ .\ m_o) / (A_o\ .\ m)$$

Determination of the Total Tannins Content

The total content of tannins was determined using FCR. About10 ml (100µg/ml) of each fraction (solution 1, S1) was mixed with 100 mg of casein with interval shaking for two hours (adsorption oftannins) and then filtered (solution 2, S2). The total phenolic contentsfor both solutions S1 and S2 using Folin–Ciocalteu's methodas (Grubesic *et al.* & Vladimir-Knezevic *et al.*, 2005). The difference between absorbances of S1 and S2 correspond to concentration casein-adsorbed tannins in sample. All determinations were carried out in triplicates. The total casein-adsorbed tannins are expressed as the number of equivalents of gallic acid.

Determination of Fatty acids

Extract was evaporated under vacuum to obtain fatty oil. A few drops of 0.5N of alcoholic potassium hydroxide was added to small quantities of the *Capparis Zeylanica root* extracts along with a drop of Phenolphthalein separately and heat on a water bath for 1-2 hrs. The formation of soap indicates the presence of Fixed oils and Fats. The hydrolyzed oil was then separated to obtain unsaponified fraction and free fatty acid. Unsaponified part was collected and further hydrolyzed to get complete free fatty acids. Hydrochloric acid water was added to make the water layer (containing the free fatty acids) acidic and extracted with diethyl ether for complete separation of free fatty acids and estimation of quantity by titration with standard alkali (I.P., 1996).

DPPH Radical Scavenging Activity

The free radical scavenging capacity of the extracts was determined using DPPH (Hasan *et al.* 2006). The DPPH solution (0.006% w/v) was prepared in 95% ethanol. The ethanol extract of the BVV leaves was mixed with 95% ethanol to prepare the stock solution (1 mg/ml). Freshly prepared DPPH solution was taken in test tubes and extracts were added followed by serial dilutions (100-1000 μg) to every test tube such that the final volume was 2 ml, and discoloration was measured at 517 nm after incubation for 30 min in the dark (Themo UV1 spectrophotometer, Thermo Electron Corporation, England, UK). Measurements were performed at least in triplicate. Ascorbic acid was used as a reference standard and dissolved in DDW to make the stock solution with the same concentration (1 mg/ml). The control sample was prepared, which contained the same volume without any extract and 95% ethanol was used as the blank.

Percent scavenging of the DPPH free radical was measured using the following equation:

DPPH scavenging effect (%) = (Ao - A1) / Ao X 100

where, Ao was the absorbance of the control and A_1 was the absorbance in the presence of the sample (ethanolic extract of *Capparis Zeylanica* root) The actual decrease in absorption induced by the test compounds was compared with the positive controls. The IC_{50} value was calculated using the dose inhibition curve.

Hydrogen Peroxide Scavenging

This activity was determined according to a previously described method with minor changes (Oyaizu, 1986). An aliquot of H_2O_2 (2 mM) and various concentrations (100-1000 μg/ml) of samples were mixed (1:0.6 v/v) and incubated for 10 min at room temperature. After incubation, the absorbance of hydrogen peroxide at 230 nm was determined against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for background subtraction. The percentage scavenging activity of hydrogen peroxide by the *Capparis Zeylanica* root extract was calculated as follows:

% scavenging activity [H_2O_2] = [Abs (control) - Abs (standard) / Abs (control)] X 100

Where, Abs (control): absorbance of the H_2O_2 (2 mM) as control

Abs (standard): absorbance of the extract / standard

Reducing Power

The reducing power of the *Capparis Zeylanica* root extract was determined according to the method previously described (Evans *et al.*, 1997). Different concentrations of the extract (250-2,500 µg) in 1 ml of double distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [K_3 $Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50ºC for 20 min. A portion (2.5 ml) of TCA (10%) was added to the mixture, which was then centrifuged at 3,000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with double distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as a reference standard. Phosphate buffer (pH 6.6) was used as a blank solution. The absorbance of the final reaction mixture of the two parallel experiments was taken and expressed as mean ± standard deviation.

Animals and treatment

Experiments were performed on adult male Wistar rats from the animal room of Institute of Medical Science, BHU, Varanasi weighing 180–200 g. They were kept under standardized conditions (temperature 21–24 °C and a light/dark cycle of 12 hours/12 hours) and fed a normal laboratory diet. After 1 week of acclimatization, rats were divided into one control and four experimental groups with 6 animals in each group. The study protocol was as per the guide line of IMS, BHU, Varanasi Ethics Committee. The extract was dissolved in normal saline to provide a 20 mg mL–1 solution. Animals from group 1 to 3 received doses expressed on the basis of mg dry extract per kg body mass, namely 100, 200, and 400 mg/kg per day of the extract by intra-gastric intubation for 14 days. Group 4 received α–tocopherol (10 mg/kg per day) dissolved in saline by intra-gastric intubation as a reference antioxidant for comparison. The fifth group of animals was treated as control and received only saline.

Blood collection

About 4 mL of blood was collected through direct heart puncture from anesthetized rats. Intraperitoneal administration of pentobarbital (60 mg/kg) was used to induce anesthesia in rats. The blood was centrifuged at 2000 × *g* for 10 minutes to separate serum. The serum was kept at –20 °C for subsequent determination of lipid peroxidation and antioxidant status.

Lipid peroxidation assay

Thiobarbituric Acid Reactive Substances (TBARS) assay is the method of choice for screening and monitoring lipid peroxidation, a major indicator of oxidative stress. To precipitate the serum proteins, 2.5 mL of TCA 20% (*m/V*) was added into 0.5 mL of the sample, which was then centrifuged at 1500 × *g* for 10 min. Then 2.5 mL of sulfuric acid (0.05 m L–1) and 2 mL TBA (0.2%) was added to the sediment, shaken, and incubated for 30 min in a boiling water bath. Then, 4mL *n*-butanol was added, and the solution was centrifuged, cooled and the supernatant absorption was recorded at 532 nm using a UV-Visible spectrophotometer (Shimadzu, Japan). The calibration curve was obtained using different concentrations of 1, 1, 3, 3-etramethoxypropane as standard to determine the concentration of TBA-MDA adducts in samples (Satho, 1978).

Total antioxidant power (TAP) assay

The total antioxidant capacity of serum was determined by measuring its ability to reduce Fe^{3+} to Fe^{2+} by the FRAP (Ferric Reducing Ability of Plasma) test. The FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue colored Fe(II)-tripyridyltriazine compound from Fe(III) by the action of electron donating antioxidants. The FRAP reagent consists of 300 mmol L–1 acetate buffer pH = 3.6, 10 mmol L–1 TPTZ in 40 mmol L–1 HCl and 20 mmol L–1 $FeCl_3$´ 6 H_2O in the ratio of 10:1:1. Briefly, 10 mL of serum was added to 300 mL freshly prepared and pre warmed (37°C) FRAP reagent in a test tube and incubated at 37°C for 10 min. The absorbance of the blue colored complex was read against a reagent blank (300 mL FRAP reagent + 10 mL distilled water) at 593 nm. Standard solutions of Fe^{2+} in the range of 100 to 1000 mmol L–1 were prepared from ferrous sulphate ($FeSO_4 \cdot 7 H_2O$) in water. The data was expressed as mmol ferric ions reduced to ferrous form per liter (FRAP value) (Benzie, 1996).

DPPH radical scavenging activity

In this test, serum ability to inhibit DPPH radical was measured (Yokozawa, 1998). DPPH is one of the few stable organic nitrogen radicals and has a maximum of absorption at 517 nm. 20 mL of rat's serum was added to 3 mL of DPPH solution (0.1 mmol L–1 in ethanol) and the reaction mixture was shaken vigorously. After incubation at room temperature for 10 min, the absorbance of this solution was determined at 517 nm DPPH solutions without serum and with α–tocopherol were used as the control and reference, respectively.

Determination of LD50

In order to determine the acute toxicity (LD_{50}) of *Capparis Zeylanica*, doses of 10, 100, 1000, and 2000 mg/kg of the day extract were administrated to rats via intragastric tube. The animals were observed for 48 h and mortality was recorded at the end of this period (Hayes, 1988).

Statistical Analysis

Results are expressed as mean ± S.E.M. of triplets. One way analysis of variance (ANOVA) was used to analyze the results with post-hoc analysis, and where appropriate, Turkey's test was employed; p values less than 0.05 were considered significant.

RESULTS

Plant Yield

The yield of the *Capparis Zeylanica* root ethanolic extract was 10.46%.

Preliminary Phytochemical Screening

Preliminary phytochemical screening of the extract revealed the presence of various bioactive components, of which phenol, fatty acids and tannins were the most prominent, and the result of the phytochemical test has been summarized in (Table 1, 2). Reports are also available where the phenolic compounds, flavonols, fatty acids and tannins are found to be associated with the antioxidative activity in biological systems (Shinde *et al.*, 2007).

DPPH Scavenging Activity of the *Capparis Zeylanica* ethanolic root Extract Compared with Standard Ascorbic Acid (*In vitro*)

In this present study, the antioxidant activity of the ethanol extracts of the *Capparis Zeylanica* root was investigated using the DPPH scavenging assay, reducing power of the extract and by determining the total antioxidant capacity of the extract. All these have proven the effectiveness of the ethanol root extract of *Capparis Zeylanica* compared with the reference standard antioxidant ascorbic acid. The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron that is responsible for the absorbance at 540 nm and also for the visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. Comparison of the antioxidant activity of the extract and ascorbic acid is shown in (Figure 1). The ethanol extract of *Capparis Zeylanica* exhibited a significant dose-dependent inhibition of DPPH activity, with a 50% inhibition (IC_{50}) at a concentration of

Table 1. Preliminary phytochemical screening of *Capparis Zeylanica* root extract

Constituent	Pet. ether	Chloroform	Ethyl acetate	Butanol	Alcohol
Alkaloids	-	+	-	-	+
Carbohydrates	-	-	-	-	-
Coumarins	-	-	-	-	-
Flavonoids	-	+	+	+	+
Fixed oils	+	-	-	-	+
Glycosides	-	-	-	-	-
Gums and resins	-	-	-	-	-
Mucilages	-	-	-	-	+
Proteins and amino acids	-	-	-	+	+
Saponins	-	-	+	+	+
Steroids and sterols	+	-	-	+	+
Tannins	+	+	+	+	+
Triterpenoids	-	-	-	+	+

Table 2. Estimation of Phytoconstituents in methanol extracts of *Capparis Zeylanica* root.

Chemical Constituents	Amount
Total Tannin	21.1±2.37 mg/g GAE/g of dry extract
Total Flavonoids	0.876±0.26 mg/g Rutin/g of dry extract
Total Flavonols	1.653 ± 1.41mg rutin equivalents/g dry extract
Total Phenols	43.625±1.45 mg/g GAE/g of dry extract
Total simple phenolic	18.875±1.33 mg/g GAE/g of dry extract
Total Saponin	1.22±0.35 mg/g of dry extract
Fatty acids	0.0867 ± 0.01 mg/g of dry extract

532 µg/ml as compared with the standard ascorbic acid (269.53 µg/ml).

Hydrogen Peroxide Scavenging

(Figure 2) shows that the root extract is a good scavenger of H_2O_2 (IC_{50} = 521.74 ± 25.61 mg/ml) compared with standard ascorbic acid (IC_{50} = 700 ± 0.3 mg/ml). The IC_{50} value (Figure 2) of the extract was lesser than that of the standard.

Reducing Power Assay

The reductive capabilities of the plant extract compared with ascorbic acid have been depicted in (Figure 3). The reducing power of the extract of *Capparis Zeylanica* root was found to be remarkable, which increased gradually with a rise in the concentration. As illustrated in (Figure 3), Fe^{3+} was transformed to Fe^{2+} in the presence of the extract and the reference compound ascorbic acid to measure the reductive capability. At 0.25 mg/ml, the absorbance of the plant extract and ascorbic acid was 0.026 and 0.016, respectively, while at 2.0 mg/ml, the absorbance of both the extract and ascorbic acid were almost the same. From the figure, it can be inferred that a low dose of the extract shows the maximum reducing capability when compared with the standard.

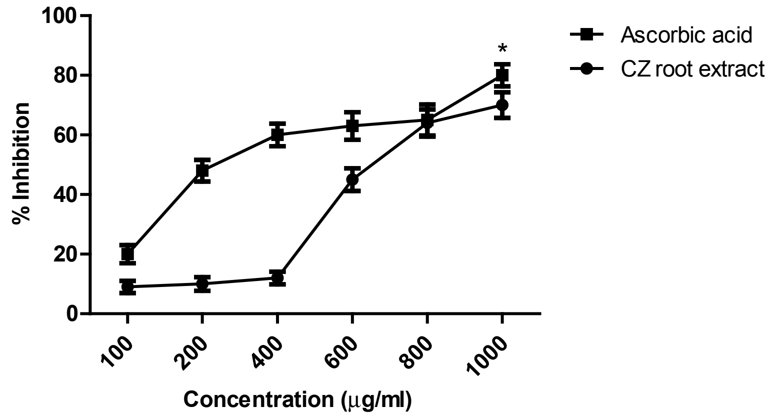

Figure 1. Inhibition v/s Concentration, of *Capparis Zeylanica*, Significantly different *P-value<0.05 from CZ group, as was determined by one-way ANOVA followed by the Turkey's test.

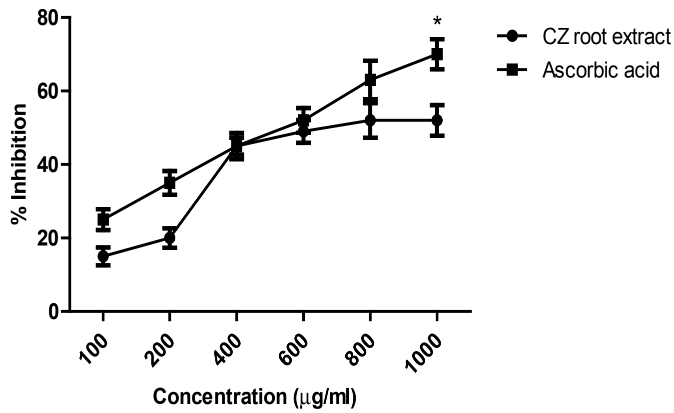

Figure 2. Hydrogen peroxide assay, Significantly different *P-value<0.05 from CZ group, as was determined by one-way ANOVA followed by the Turkey's test.

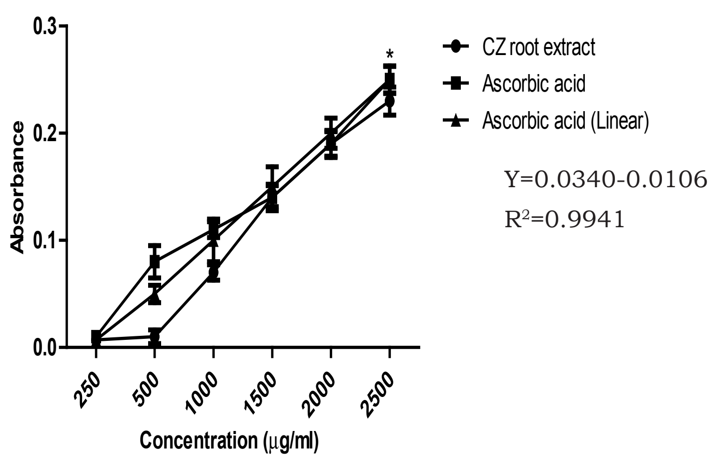

Figure 3. Reducing power assay, Significantly different *P-value<0.05 from CZ group, as was determined by one-way ANOVA followed by the Turkey's test.

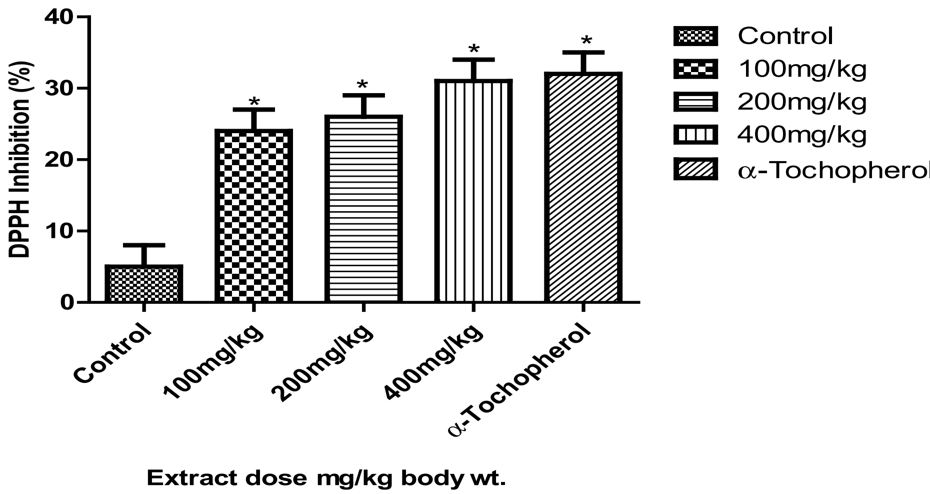

Fig. 4 (a)

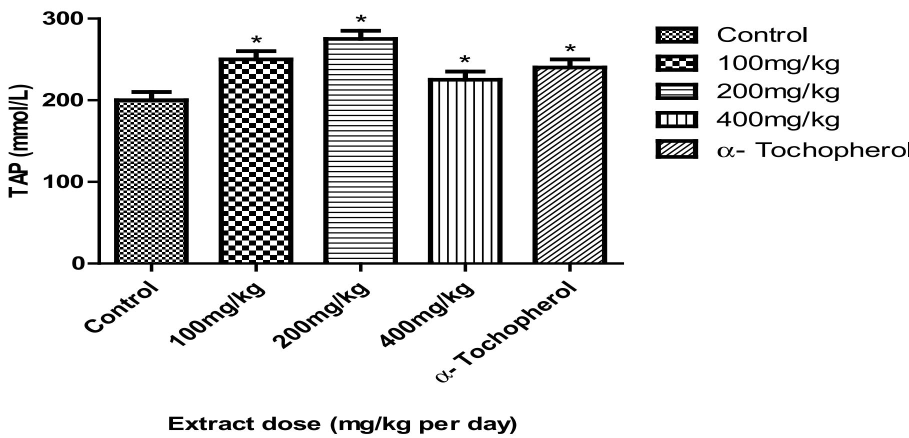

Fig. 4 (b)

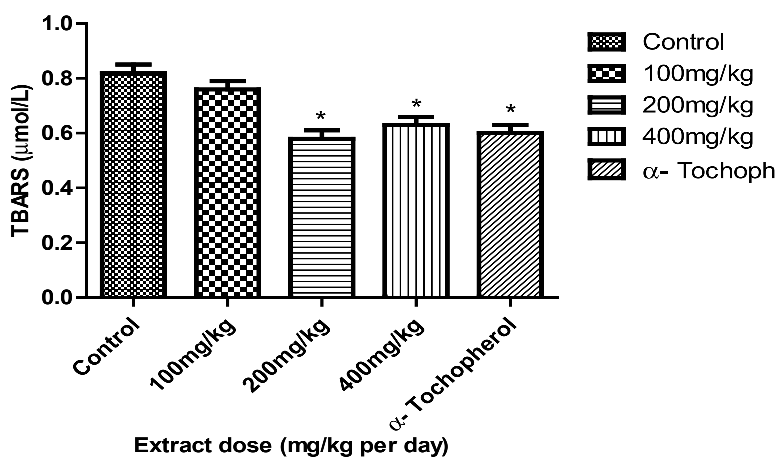

Fig. 4 (c)

Fig. 4. Antioxidant potential of CZ in assays: a) DPPH, b) TAP and c) TBARS compared to α-tocopherol in rat blood. Data are mean ± SEM of 6 animals in each group. *Different from the respective control ($p < 0.05$) followed by Turkey's test. α-Tocopherol was administered at a dose of 10 mg/kg per day.

Determination of LD_{50} (*In vivo*)

The acute toxicity test (LD_{50}) demonstrated that *Capparis Zeylanica* extract is not lethal up to a dose of 2000 mg/kg.

DPPH radical scavenging activity

Capparis Zeylanica extracts in all doses (mg/kg) used significantly ($p < 0.05$) increased the serum DPPH scavenging potential when compared to the control as follows: 100 (480%), 200 (520%), 400 (620%). This value for α–tocopherol (10 mg/kg) compared to the control was 640% ($p < 0.05$) (Fig. 4a).

Total antioxidant power (TAP) assay

Capparis Zeylanica extract in the all doses (mg/kg), significantly ($p < 0.005$) increased the serum TAP when compared to the control as follows: 100 (25.0%), 200 (37.5%), 400 (12.5%) (Fig. 4b). This value for α–tocopherol (10 mg/kg) compared to the control in the TAP assay was 20.0% ($p < 0.05$) and at dose of 400 mg/kg per day did not significantly alter the serum TAP.

Lipid peroxidation assay

Capparis Zeylanica extract in the same doses (mg/kg), significantly ($p < 0.005$) decreased the serum TBARS when compared to the control as follows: 100 (7.3%), 200 (29.3%), 400 (23.2%) (Fig.4c). This value for α–tocopherol (10 mg/kg) compared to the control in the TBARS assay 26.8% ($p < 0.05$) and at dose of 400 mg/kg per day did not significantly alter the serum TBARS.

DISCUSSION

Medicinal plants have become extremely popular all over the world as antioxidants and markets are flooded with herbal formulations. Studies in the USA have reported that roughly 40–60% of cancer patients who use some form of complementary medicine include the use of herbs, vitamins, antioxidants or all three (Wang *et al.*, 2005). Free radicals are involved in the normal physiology of living organisms. Under certain conditions, the excess of free radicals and reactive oxygen species have been proposed to induce cellular damage and to be involved in several human diseases such as cancer, arteriosclerosis, inflammatory disorders as well as in ageing process. In recent past several dietary and herbal formulations which have free radical scavenging potential have gained important in treating such chronic diseases (Tiwari *et al.*, 2007). In the present experiment, the radical scavenging activity of ethanol extract of *Capparis Zeylanica* root growing in India is estimated using DPPH assay. Antioxidant studies indicate that *Capparis Zeylanica* root extract possess the ability of either inhibiting free radical formation or itself be a free radical scavenger. The ethanolic extract of *Capparis Zeylanica* root showing almost similar response as reported earlier (Arol *et al.*, 2010). The dose inhibition curve and IC_{50} values of the root extract are shown in [Figure 1]. In the dose-response experiment, it could be observed that total inhibition of the enzymes was never achieved. The maximum inhibition was in the range of 75-85% in the presence of the 25 mg/ml extract. With the addition of a larger amount of extract to the DPPH assay mixture, the degree of inhibition decreased, indicating a pro-oxidant effect. The explanation for the higher IC_{50} (IC_{50} = 269.53 μg/ml) value found in the experiment was because the sample used was a crude extract, with the compound(s) reacting as antioxidants.

The antioxidative effect of *Capparis Zeylanica* root extract is mainly due to phenolic components such as tannins and phenols (Zhang *et al.*, 2005). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Osawa T, 1994). In this respect, polyphenolic compounds like tanins and phenols commonly found in plants have been reported to have multiple biological effects, including an antioxidant activity (Gil *et al.*, 1999 & Vinson *et al.*, 1995). For measurements of the 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. Similar trends have been observed with the *Capparis Zeylanica* root extract. Earlier authors have observed a direct correlation between antioxidant activity and reducing power of certain plant extracts (Tanaka *et al.*, 1988). The presence of reducing agents is thought to be associated with the reducing properties (Duh *et al.*, 1999), which in turn have been shown to exert an antioxidant action by donating a hydrogen atom that breaks the free radical chain (Gordon, 1990).

Data obtained by DPPH, FRAP, and TBARS assays indicate that *Capparis Zeylanica* effectively inhibits oxidative stress *in vivo*. When tested by FRAP and TBARS, *Capparis Zeylanica* extract at a dose of 400 mg/kg per day exhibited no significant antioxidant activity in comparison to the control. It is proposed that the DPPH test is more sensitive than FRAP and TBARS tests for examination of the antioxidant capacity of *Capparis Zeylanica*. The ethanolic extract of *Capparis Zeylanica* root showing almost similar response as reported earlier (Arol PM, 2010) for *in vitro* study. The antioxidative effect of *Capparis Zeylanica* root extract is mainly due to phenolic components such as tannins and phenols (Zhang *et al.*, 2005). The *Capparis Zeylanica* extract also consists of fatty acids containing the conjugated double bonds like linoleic acids, palmitic acids and other long chain fatty acids which are reported to be responsible for antioxidant activity. Reducing agents are also reported to react with certain precursors of peroxide thus preventing peroxide formation.

CONCLUSION

The *Capparis Zeylanica* root ethanolic extract showed a strong antioxidant activity by inhibiting DPPH, hydrogen peroxide and reducing power activities when compared with the standard L-ascorbic acid and α–tochopherol. In addition, the *Capparis Zeylanica* root was found to contain a noticeable amount of total phenols and flavonoids, which play a major role in controlling oxidation. The results of this study show that the *Capparis Zeylanica* root can be used as an easily accessible source of natural antioxidant. However, the phytoconstituents responsible for the antioxidant activity of *Capparis Zeylanica* are not much clear. Therefore, a further study is needed to determine the mechanism behind the antioxidant activity of this plant. The study indicates the interesting anti oxidative stress potential of *Capparis Zeylanica in vivo* that is comparable to that of α–tocopherol. Further studies are needed to elucidate whether *Capparis Zeylanica* root could be useful in the management of human diseases resulting from oxidative stress.

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