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Antioxidant and free radical scavenging activities of *Cleome rutidosperma*

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SUMMARY

The study was aimed at evaluating the antioxidant and free radical scavenging activities of ethanolic extract and its fractions of *Cleome rutidosperma***.** The antioxidant activity, reducing power, total phenolic content, total flavonoid content, superoxide anion scavenging activity, nitric oxide anion scavenging activity, *in vitro* antilipid peroxidation activity and *in vitro* non-enzymatic hemoglobin glycosylation were studied**.** The results obtained in the study indicate that *Cleome rutidosperma* is a potential source of natural antioxidant. All the parameters were found to be concentration dependent and increased with increasing amounts of sample. Flavonoids, phenolic compound like tannins, terpenoids may be responsible for the antioxidant activity of the plant. Variation of solubility parameters in various models may be attributed to non-linearity of activity of ethanol extract fractions models. Further investigation on the isolation and identification of antioxidant component(s) in the plant may lead to chemical entities with potential for clinical use.

Key words: *Cleome rutidosperma*; *In vitro*; Antioxidant activity; Free radical scavenging activity

INTRODUCTION

Oxidative stress plays an important role in the pathogenesis of various diseases such as atherosclerosis, alcoholic liver cirrhosis and cancer etc (Freeman and Crapo, 1982; Maxwell and Lip, 1997). Oxidative stress is initiated by reactive oxygen species (ROS), such as superoxide anion (O^{-}_{2}), perhydroxy radical ($HOO^{\cdot}$) and hydroxyl radical (HO). These radicals are formed by one-electron reduction process of molecular oxygen (O_2). ROS can easily initiate the lipid peroxidation of the membrane lipids, causing damage of the cell membrane of phospholipids, lipoprotein by propagating a chain reaction cycle (Pryor, 1973). Thus, antioxidants defense systems have coevolved with aerobic metabolism to counteract oxidative damage from ROS.

The role of free radicals in many disease conditions has been well established. Several biochemical reactions in our body generate reactive oxygen species and these are capable of damaging crucial bio-molecules. If cellular constituents do not effectively scavenge them, they lead to disease conditions (Halliwell and Gutteridge, 1985; Halliwell, 1994).

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Further, recent investigations have shown that the antioxidant properties of plants could be correlated with oxidative stress defense and different human diseases like aging process etc (Stajner *et al.*, 1998; Sanchez-Moreno *et al.*, 1999; Malencic *et al.*, 2000 Mukherjee, 2002). In recent years one of the areas which attracted a great deal of attention is antioxidants in the control of degenerative diseases in which oxidative damage has been implicated. Several plant extracts and different classes of phytochemicals have been shown to have antioxidant activity (Freeman and Crapo, 1988; Rao, 1997; Tripathi *et al.*, 1997; Vanil *et al.*, 1997).

Cleome rutidosperma (Capparidaceae) is a low-growing herb, up to 70 cm tall, found in waste grounds and grassy places with trifoliate leaves and small, violet-blue flowers, which turn pink as they age. The elongated capsules display the asymmetrical, dull black seeds. The plant is native to West Africa, although it has become naturalized in various parts of tropical America as well as Southeast Asia (Widespread, 1972; Waterhouse and Mitchell, 1998). According to traditional use, the different parts of the plants of *Cleome* genus are used as stimulant, antiscorbutic, anthelmintic, and rubifacient, vesicant and carminative (Kiritikar and Basu, 1991). The antiplasmodial, analgesic, locomotor, antimicrobial, diuretic, laxative activities of *Cleome rutidosperma* were reported earlier (Bidla *et al.*, 2004; Bose *et al.*, 2004; Bose *et al.*, 2005; Bose *et al.*, 2006).

Although, no work regarding evaluation of antioxidant activity has been reported on *Cleome rutidosperma*, similar works have been reported on *Cleome pentaphylla*, another locally available plant in tropical part of India of the same genus (Kumar and Sadique, 1987; Cooks *et al.*, 1998). In the present study, we report the antioxidant activity of ethanolic extract of aerial parts of *Cleome rutidosperma*.

MATERIALS AND METHODS

Plant materials

The Plant material (whole plant) was collected from North 24-Pargana district of West Bengal, India during September 2005 and was authenticated at Botanical Survey of India, Shibpur, Howrah and West Bengal, India. A voucher specimen (*C.R.-1*) has been kept in our research laboratory for future reference. The fresh aerial parts were washed under running tap water to remove adhered dirt, followed by rinsing with distilled water, shade dried and pulverized in a mechanical grinder to obtain coarse powder.

Preparation of extracts

The aerial parts were extracted with 90% ethanol using Soxhlet apparatus. The solvent was removed under reduced pressure, which gave a greenish-black coloured sticky residue (yield- 11.6% w/w on dried material basis). A portion of dried ethanolic extract was suspended in water and fractionated successively with petroleum ether (40 - 60×C), diethyl ether, ethyl acetate and n-butanol. All the fractions were dried by distillation under reduced pressure. Standard methods (Trease and Evans, 1989; Harborne, 1994) were used for preliminary phytochemical screening of the ethanolic extract and its fractions to know the nature of phytoconstituents present in it.

Evaluation of antioxidant and free radical scavenging activities

Evaluation of antioxidant activity

The antioxidant activity of extracts was evaluated by the formation of phosphomolybdenum complex method, according to Prieto *et al.* (1999). In this method, an aliquot of 0.4 ml of sample solution (100 ppm in methanol) was mixed in a vial with 4 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The blank was prepared by replacing the sample with 0.4 ml of methanol. The vials were capped and incubated in a water bath at 95°C for 90 min. After cooling the samples at room temperature, the absorbances were measured at 695 nm against the blank. The antioxidant activity

was expressed relative to that of ascorbic acid.

Reducing power

The reducing power of *C. rutidosperma* was determined according to the method of Oyaizu, 1986. Briefly, 10 mg of *C. rutidosperma* extract in 1 ml distill water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%). The mixture was incubated at 50 °C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3,000 rpm for 20 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. The activity was compared with butylated hydroxy toluene (BHT) standard.

Determination of total phenolic compounds

Total soluble phenolics in the ethanolic extract and its fractions of *C. rutidosperma* were determined with Folin Ciocalteu reagent using pyrocatechol as a standard (Slinkard and Singleton, 1977). Briefly, 0.1 ml of extract solution (contain 1000 μg extract) in a volumetric flask was diluted in distilled water (46 ml). About 1 ml of Folin Ciocalteu reagent was added and the content of the flask was mixed thoroughly. After 30 min, 3 ml sodium carbonate was added then the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm. The concentration of total phenolic compounds in the *C. rutidosperma* determine as microgram (μg) of pyrocatecol equivalent by using an equation that was obtained from standard pyrocatecol graph. The equation for calculating the pyrocatecol was:

Absorbance = 0.001 × pyrocatecol (μg) - 0.003.

Total flavonoids determination

Aluminum chloride colorimetric method was used for flavonoid content determination (Chang, *et al*., 2002). Each extract (0.5 ml of 1: 10 g/ml) in methanol was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 μg/ml in methanol.

Superoxide anion scavenging activity

Measurement of super oxide anion scavenging activity of *C. rutidosperma* extracts were based on the capacity of the extracts to inhibit formazon formation by scavenging the superoxide radical generated in riboflavin-light-NBT system (Beauchamp and Fridovich, 1971; Ravishankara, 2002). The reaction mixture contained 50 mM phosphate buffer pH 7.6, 20 μg riboflavin, 12 mM EDTA, NBT 0.1 mg/3 ml, added in that sequence. Reaction was started by illuminating the reaction mixture containing different concentrations of sample extract for 90 s and the absorbance was measured immediately at 590 nm. Ascorbic acid was used as a positive control. Decreased absorbance of the reaction mixture indicated increase superoxide anion scavenging activity.

Nitric oxide anion scavenging activity

The procedure is based on the principal that, sodium nitropruside solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitric ion that can be estimated using Greiss reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% naphthylenediamine dihydrochloride). Scavenger of nitric oxide competes with oxygen, leading to reduce production of nitric ion (Rao, 1997).

For the experiment, sodium nitropruside (10 mM) in phosphate buffered saline was mixed with different concentration of ethanolic extract and its fractions dissolved in ethanol and incubated at room temperature for 150 min. The reaction

without the extract sample but equivalent amount of ethanol served as control. After incubation period, 0.5 ml of Greiss reagent was added. The absorbance of the chromophore formed was read at 546 nm. Ascorbic acid was used as positive control.

***In vitro* antilipid peroxidation activity**

In the experiment, the reaction mixture containing rat liver homogenate (0.1 ml, 25% w/v) in Tris-HCl (30 mM), ferrous ammonium sulphate (0.16 mM), ascorbic acid (0.06 M) and different concentration of the extracts (from 10 to 1,000 μg/ml) in a final volume of 0.5 ml was incubated for 1 h at 37 °C (Prieto *et al.,* 1999) and the resulting thiobarbituric reacting substance (TBARS) was measured (Ohhkawa *et al*., 1979). In this method, 0.4 ml of the reaction mixture was treated with sodium dodecyl sulfate (0.2 ml, 8.1%) and acetic acid (1.5 ml, 20%, pH 3.5) made to a total volume of 4 ml by addiding distilled water and kept in water bath at 95 °C for 1 h. After cooling distilled water (1 ml) and 5 ml of n-BuOH / pyridine (15: 1) (v/ v) were added. After shaking and centrifugation, the organic layer was separated and the absorbance measured at 532 nm.

***In vitro* non-enzymatic haemoglobin glycosylation**

In vitro non-enzymatic hemoglobin glycosylation was measured by method of Yadav *et al*. (2000). In this method, 5 g% haemoglobin, in 0.01 M phosphate buffer (pH 7.4) was incubated in presence of 2 g/ 100 ml concentration of glucose for 72 h in order to find out the best condition for hemoglobin glycosylation. The assay was perform by adding 1 ml of glucose solution, 1 ml of hemoglobin solution and 1ml gentamycin (20 mg/100 ml) in 0.01 M phosphate buffer (pH 7.4) with various concentrations of samples. The mixtures were incubated in dark at room temperature. The degree of glycosylation of haemoglobin in the presence of different concentration of fractions and their absorbance were measured colorimetrically.

Data analysis

Data presented are means ± standard deviation for three replicates. The absorbance readings obtained in various models were compared to control readings using student's *t*-test (with significance at $P < 0.05$).

RESULTS

The antioxidant activity of the ethanolic extract and its fractions of C*. rutidosperma* were evaluated in different *in vitro* models. The results of various models are summarized in Table 1. Statistical analysis indicated significant activities of all extracts tested (excepting a few cases of low concentrations) with $P < 0.05$. Further, the same ethanolic extract and its fractions were subjected to preliminary phytochemical screening for the presence of different chemical groups (Table 2).

Fig. 1 shows the reductive capabilities of the plant extracts in terms of ascorbic acid equivalent. The plant ethanolic extract was found to have total ascorbic acid content of 83.43 ± 7.87 mg ascorbic acid equivalent / g extract. The order of activity of various fractions were pet-ether fraction > n-butanol fraction > diethyl ether fraction > ethyl acetate fraction.

Fig. 2 shows the reductive capabilities of the plant extract compared to BHT. The reducing power of ethanolic extract and its fractions of *C. rutidosperma* were found to be potent and the power increased with quantity of sample. The plant extract and its fractions could reduce the most Fe^{3+} ions, although which had a lesser reductive activity than the standard.

The total phenolic contents of ethanolic extract and its fractions of *C. rutidosperma* are shown in Fig. 3. The plant ethanolic extract was found to have total phenolic content of 73.37 ± 10.28 μg pyrocatecol equivalent / 500 mg extract. The total phenolic content of extract fractions varied from 83.37 ± 8.29 to 208.37 ± 18.43 μg pyrocatecol equivalent / 500 mg extract.

Fig. 4 indicates the total flavonoid contents of

Table 1. Antioxidant and free radical scavenging activities of *C. rutidosperma*

Extract	Antioxidant activity[a]	Total Phenolic Content[b]	Total Flavonoid Content[c]	EC_{50} Super oxide scavenging activity (μg/ml)	EC_{50} Nitric oxide scavenging activity (μg/ml)	EC_{50} *Invitro* Lipid peroxidation (μg/ml)	EC_{50} *Invitro* Haemoglobin Glycolisation (μg/ml)
Ethanol extract	83.43 ± 7.87	73.37 ± 10.28	5.36 ± 0.38	59.2	52.51	97.88	14.93
Pet ether fraction	150.86 ± 9.95	83.37 ± 8.29	0.79 ± 0.03	59.64	42.95	56.99	6.08
Diethyl ether fraction	84.96 ± 8.56	175.03 ± 17.30	1.59 ± 0.08	38.3	78.47	79.6	5.66
Ethyl acetate fraction	55.08 ± 6.83	149.70 ± 10.81	6.95 ± 0.22	71.27	86.76	79.37	9.94
n-Butanol fraction	96.55 ± 8.04	208.37 ± 18.43	11.19 ± 0.45	35.39	54.55	46.96	8.98
Standard 1	-	-	-	37.74 (Ascorbic acid)[d]	23.89 (Ascorbic acid)[d]	49.96 (α-tocopherol)[d]	7.42 (α-tocopherol)[d]
Standard 2		-	-	-	-	-	11.72 (Rutin)[d]

All values are expressed as mean ± S.D. (n = 3). [a]mg equivalent of ascorbic acid per gram, [b]μg of pyrocatechol equivalent per 500 mg, [c]mg equivalent of quercetin per gram. Names in the parenthesis indicate the respective standard used.

Table 2. Phytochemical screening of extracts of *C. rutidosperma* aerial parts

Extract	Phytoconstituents present
Ethanolic extract	Lipids, steroids, terpenoids, flavonoids, tannins, saponins, sugars
Pet-ether fraction	Lipids, steroids, terpenoids
Diethyl ether fraction	Steroids, terpenoids, flavonoids
Ethyl acetate fraction	Flavonoids, tannins, saponins
n-Butanol fraction	Flavonoids, tannins, saponins

ethanolic extract and its fractions of *C. rutidosperma*. The ethanolic extract was found to have total flavonoid content of 5.36 ± 0.38 mg quercetin equivalent / gm extract. The total flavonoid content of extract fractions ranged from 0.79 ± 0.03 to 11.19 ± 0.45 mg quercetin equivalent / gm extract. The n-butanol fraction was found to have highest flavonoid content whereas pet-ether fraction was found to contain least.

The scavenging of super oxide by plant extract and its fractions were increased in a dose-dependent manner (Fig. 5). The EC_{50} value of ethanol extract was observed to be 59.2 μg / ml. The IC50 values of pet-ether, diethyl ether, ethyl acetate, n-butanol fractions were respectively found to be 59.64, 38.3, 71.27 and 35.39 μg/ml. These EC_{50} values of the standard, ascorbic acid was found to be 37.74 μg/ml.

The scavenging of nitric oxide by plant extract and its fractions were increased in a dose-dependent manner as illustrated in figure 6. At concentration of 52.51 μg/ml of ethanol extract 50% of nitric oxide generated by incubation was scavenged. The EC_{50} values of pet-ether, diethyl ether, ethyl acetate, n-butanol fractions were respectively found to be 42.95, 78.47, 86.76 and 54.55 μg/ml. These IC_{50}

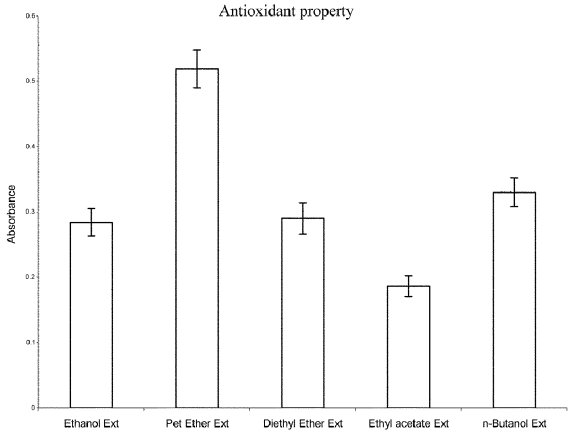

Fig. 1. The antioxidant activity of ethanolic extract of *Cleome rutidosperma* and its fractions. Results are mean ± S.D. of three parallel measurements.

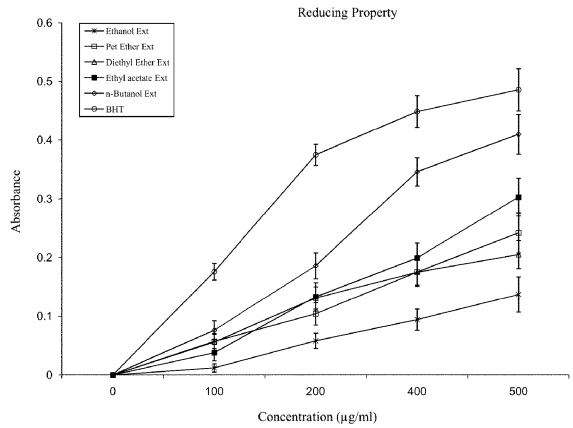

Fig. 2. The reductive ability of ethanolic extract of *Cleome rutidosperma,* its fractions and BHT. Results are mean ± S.D. of three parallel measurements.

values were found to be lesser than the standard, ascorbic acid (EC_{50} 23.89 µg/ml).

Activity of plant extract against non-enzymatic lipid per-oxidation in rat liver microsomes has been shown in Fig. 7. Additions of Fe^{2+}/ascorbate to the liver microsomes cause increase in lipid peroxidation. The ethanol extract showed inhibition of peroxidation effect in all concentrations, which showed 50% inhibition effect at 97.88 µg/ml. The EC_{50} values of pet-ether, diethyl ether, ethyl acetate, n-butanol fractions were respectively found to be 56.99, 79.6, 79.37and 46.96 µg/ml. These inhibition

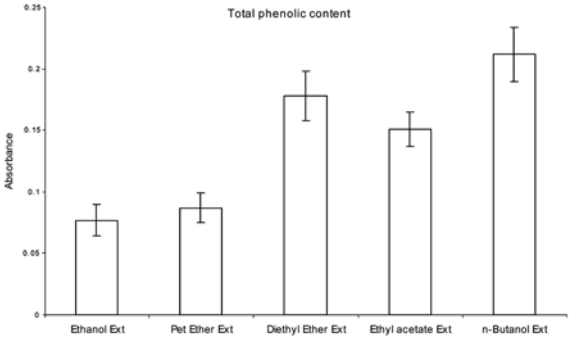

Fig. 3. The total phenolic content of ethanolic extract of *Cleome rutidosperma* and its fractions. Results are mean ± S.D. of three parallel measurements.

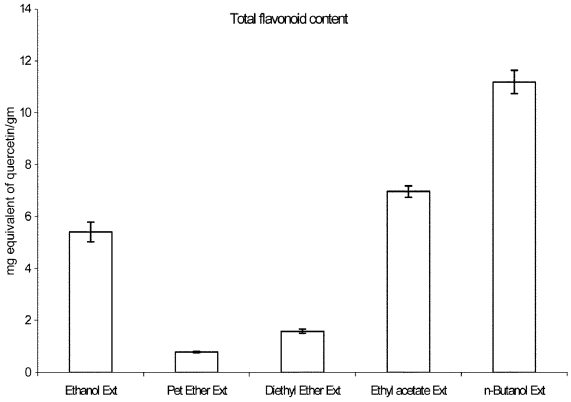

Fig. 4. The total flavonoid content of ethanolic extract of *Cleome rutidosperma* and its fractions. Results are mean ± S.D. of three parallel measurements.

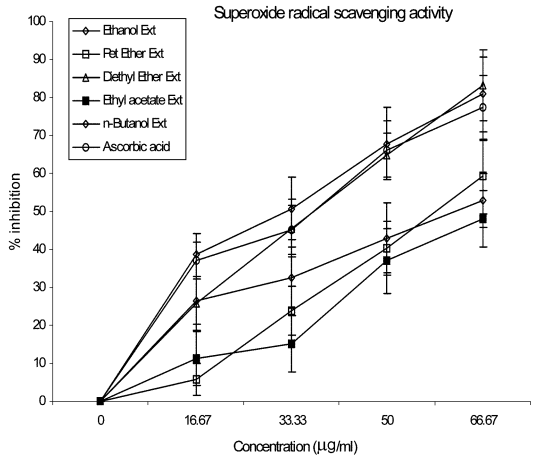

Fig. 5. Effect of ethanolic extract of *Cleome rutidosperma*, its fractions and ascorbic acid on scavenging of superoxide anion radical formation. Results are mean ± S.D. of three parallel measurements.

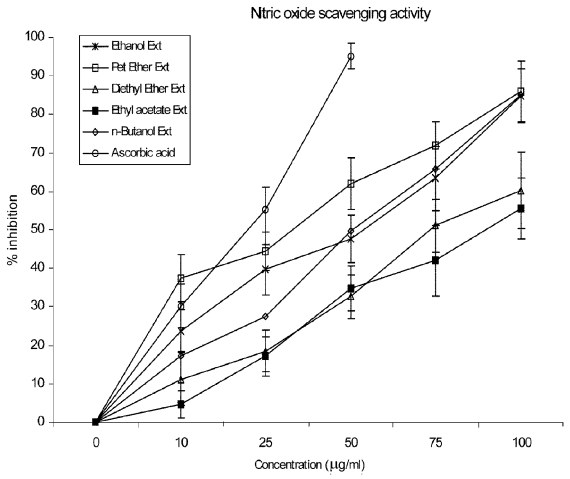

Fig. 6. Scavenging effect of ethanolic extract of *Cleome rutidosperma*, its fractions and standard ascorbic acid on Nitric oxide radical. Results are mean ± S.D. of three parallel measurements.

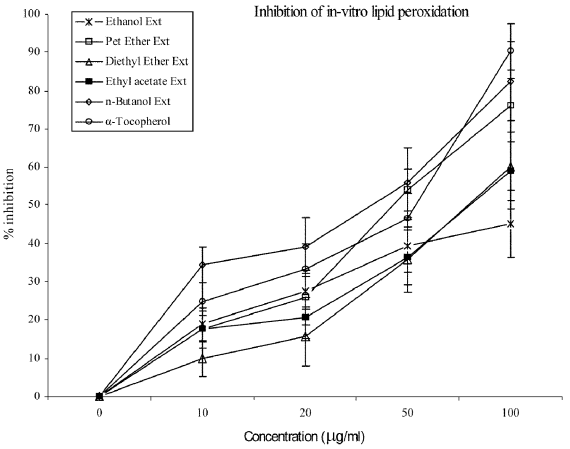

Fig. 7. Effect of ethanolic extract of *Cleome rutidosperma*, its fractions and α-tocopherol on lipid peroxidation of liver microsome induced by Fe^{2+}/ ascorbate. Results are mean ± S.D. of three parallel measurements.

values were found to be lesser than the standard, α-tocopherol (EC_{50}, 49.96 µg/ml).

The in vitro non-enzymatic haemoglobin glycosylation by plant ethanolic extract and its fractions were increased in a dose-dependent manner (Fig. 8). At concentration of 14.93 µg/ml, ethanol extract exerted 50% haemoglobin glycosylation. The EC_{50} values of pet-ether, diethyl

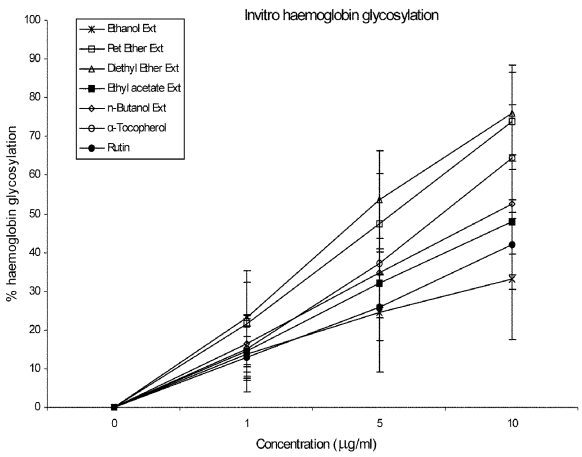

Fig. 8. Effect of ethanolic extract of *Cleome rutidosperma*, its fractions, α-tocopherol and rutin on *in vitro* non-enzymatic haemoglobin glycosylation. Results are mean ± S.D. of three parallel measurements.

ether, ethyl acetate, n-butanol fractions were respectively found to be 6.08, 5.66, 9.94 and 8.98 µg/ml. These EC_{50} values of the standards, α-tocopherol and rutin were found to be 7.42 and 11.72 µg/ml, respectively.

DISCUSSION

Free radicals have been implicated in many disease conditions, the important ones being superoxide radical, hydroxy radical, peroxyl radical and singlet oxygen. Herbal drugs containing radical scavengers are gaining importance in treating such diseases. Many plants exhibit efficient antioxidant properties owing to their phenolic and flavonoid constituents (Larson, 1988).

In the present study, We investigated the antioxidant activity of the ethanolic extract and its fractions of *C. rutidosperma* and the possible mechanism involved, basing on the response obtained in the different *in vitro* models covering major radicals *viz.*, superoxide, hydroxyl and nitric oxide radicals.

Total antioxidant activity of ethanolic extract of *C. rutidosperma* and its fractions were measured, based on the reduction of Mo (VI) to Mo (V) by the

extract and subsequent formation of green phosphate / Mo (V) complex at acidic pH. The values were expressed in terms of ascorbic acid equivalent.

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Mier *et al*., 1995). However, the antioxidant activity of putative antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides. Prevention of continued hydrogen abstraction, and radical scavenging (Hatano *et al*., 1989; Diplock, 1997). The reductive capabilities of samples of *C. rutidosperma* were compared with BHT. For the measurements of the reductive ability, we investigated the Fe^{3+} to Fe^{2+} transformation in the presence of the ethanolic extract and its fractions of *C. rutidosperma* using the method of Oyaizu, 1986.

Phenols are very important plant constituent because of their scavenging ability due to their hydroxyl group (Hatano *et al*., 1989). The phenolic compound may contribute directly to antioxidative action. It suggests that polyphenolic compounds have inhibitory effect on mutagenesis and carcinogenesis in humans. As ethanolic extract of *C. rutidosperma* and its fractions contained considerable amount of phenolic compounds, polyphenols present in them may be partly responsible for their antioxidant activity.

It has been recognized that flavonoids show antioxidant activity and their effects on human health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process (39, 40). The flavonoids, which contain hydroxyls, which are responsible for the radical scavenging effect in the plants (41, 42). The flavonoid contents of the extracts were expressed in terms of quercetin equivalent (the standard curve equation: $y = 0.0067 x + 0.0132$).

Superoxide radical is known to be very harmful to cellular components as a precursor of more reactive oxygen species (Halliwell and Gutteridge, 1985). The extract was found to be an efficient scavenger of super-oxide radical generated in riboflavin-NBT-light system in vitro and its activity was comparable to that of ascorbic acid.

Nitric oxide (NO) exhibits numerous physiological properties and it is also implicated in several pathological states (Moncada *et al*., 1991). It is an important second messenger, act as a neuro transmitter, and play an important role in the defense against pathogens as well as in the control of blood pressure. NO is produced in various cell including neurons, endothelial cell and neutrophill by three isoforms of nitric oxide synthetase enzyme, from nitrogen of guanidine group of L-arginine and from molecular oxygen (Beheti *et al*., 2005). The interaction of NO with other radical leads to formation of more hazardous radical such as peroxy nitrite anion and hydroxyl radical. In fact, NO react more rapidly with superoxide, than the latter does with superoxide dismutase. In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions (Moncada *et al*., 1991). The extracts showed a moderate nitric oxide scavenging activity.

Hydroxyl radicals are most reactive species, initiating the peroxidation of the cell membrane (Halliwell and Gutteridge, 1985). The lipid radical, thus generate would initiate chain reaction in the presence of oxygen, giving rise to lipid peroxide, which break down to aldehydes such as malondialdehyde, which are known to be mutagenic and carcinogenic (Miyake and Shibamoto, 1997). In the present study, the ethanolic extract and its fractions showed potent inhibition of lipid peroxide.

Since non-enzymatic glycosylation of haemoglobin is an oxidation reaction (Larson, 1988), an oxidation is expected to inhibit this reaction. The degree of glycosylation of haemoglobin *in vitro* can be measured colorimetrically (Fluckiger and Winterhalter, 1978). The ethanolic extract and its fractions showed potent haemoglobin glycosylation.

These results may suggest that although there is indication potential antioxidant activity of

ethanolic extract and its fractions of *C. rutidosperma*, although linear correlation is absent between various models studied.

It is reported that, the antioxidant activity of putative antioxidants have been attributed to various mechanisms: among these are prevention of chain reaction, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continuous hydrogen abstraction and radical scavenging (Diplock, 1997; Heinoman *et al.*, 1998). On the other side, solubility of a compound in the reaction environment may affect its activity (Cakir, 2003). In general the solubility of aglycone is more in nonpolar medium, while the solubility of the glycones may be higher in polar solvents. Variation of solubility parameters in various models may be attributed to non-linearity of activity of ethanol extract fractions in various models.

Preliminary phytochemical studies of the extract indicated presence of phenols, flavonoids, terpenoids, steroids, and saponins. Flavonoids, phenolic compound like tannins are the major constituents of most of the plant reported to possess antioxidant and free radical scavenging activity (Formica and Regelson, 1995; Beninger and Hosfield, 2003). Some terpenoids have also reported to have similar activities (Zhu *et al.*, 1999; Montilla *et al.*, 2003). Presence of above constituents in *C. rutidosperma* may be responsible for the antioxidant activity of the plant. Further studies to isolate the phytoconstituents responsible for the observed activity are under process.

CONCLUSION

This study suggested that the ethanolic extract and its fractions of *C. rutidosperma* possess antioxidant activity, which might be helpful in preventing or slowing the progress of various oxidative stress related diseases. Further investigation on the isolation and identification of antioxidant component(s) in the plant may lead to chemical entities with potential for clinical use.

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