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In vitro and *in vivo* Antioxidant and Toxicity Evaluation of Different Fractions of *Oxalis corniculata* Linn.

[1]M. Badrul Alam, [1]M. Sarowar Hossain, [2]Nargis Sultana Chowdhury, [3]M. Ehsanul Haque Mazumder, [4]M. Ekramul Haque and [5]Anwarul Islam

[1]Department of Pharmacy, Atish Dipankar University of Science and Technology, Dhaka, Bangladesh
[2]Department of Pharmacy, Manarat International University, Dhaka, Bangladesh
[3]Discipline of Biomedical Science, Faculty of Medicine, Cumberland Campus, University of Sydney, Australia
[4]Department of Pharmacy, BRAC University, Dhaka, Bangladesh
[5]Department of Pharmacy, Bangladesh University, Dhaka, Bangladesh

Corresponding Author: M. Sarowar Hossain, Department of Pharmacy, Atish Dipankar University of Science and Technology, House No.83, Road No. 4, Block No. B, Banani, Dhaka-1213, Bangladesh Tel: 8801717671781

ABSTRACT

In course of investigation on natural antioxidants, the present study was aimed to report the antioxidant activities, both *in vitro* and *in vivo*, of the crude methanolic extracts of the whole plant of *Oxalis corniculata* Linn along with its various organic fractions. The different assay methods, including total antioxidant activity, scavenging free radical, authentic peroxynitrite, nitric oxide and reducing power assessment were used to evaluate the antioxidant potential of the crude extract and its organic fractions. The ethylacetate (EtOAc) fraction, showed strong activity in all the model systems tested and in peroxynitrite model this fraction (IC_{50} value of 2.29±0.18 μg mL^{-1}) exerted three-fold stronger activity than standard penicillamine (IC_{50} value of 6.20±0.32 μg mL^{-1}). The reducing power of the extract was found to be concentration dependent. The administration of the extract/fractions at a dose of 250 and 500 mg kg^{-1} body weight to the male Wistar rats increased the percentage inhibition of reduced glutathione, superoxide dismutase and catalase significantly. Whereas, lipid peroxidation level in hepatotoxic rats markedly decreased at a dose of 500 mg kg^{-1} body weight after 7 days. The total phenol and flavonoid content were also measured in the crude extract along with its organic fractions. The Brine shrimp lethality bioassay was used to determine the toxicity of the extracts and Vincristin sulphate was used as positive control. The dichloromethane (CH_2Cl_2) fraction showed highest activity (LC_{50} value of 29.02±1.16 μg mL^{-1}) and other showed activity in the order of: EtOAc fraction >n-BuOH fraction> MeOH extract > aqueous fraction. Taken together, these results suggest that *O. corniculata* extract has strong antioxidant properties and further validate the traditional use of this plant.

Key words: Antioxidant enzyme, *Oxalis corniculata*, free radical, phenolic content

INTRODUCTION

Oxidation of food boosts up energy production for living beings. However, paradoxically Reactive Oxygen Species (ROS) are formed as by products including superoxide anion radical ($\bullet O^{2-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($\bullet OH$), singlet oxygen ($^1O_2$) and free radicals of lipids such as alkoxyl radical ($RO\bullet$) and peroxyl radical ($ROO\bullet$) (Gulcin *et al*., 2002;

Yildirim *et al*., 2000). In addition, Peroxynitrite ($ONOO^-$), the reactive nitrogen species (RNS), a product of the reaction of nitric oxide (NO) with superoxide anion (O^{-2}), is formed within tissues with perfusion injury and inflammation. These ROS and RNS, the main reprobate, are capable of damaging several cellular components such as proteins, lipids and DNA (Koneru *et al*., 2011). Also, these reactive species are likely to be involved in diseases such as Alzheimers disease and cancer, aging, arteriosclerosis, rheumatoid arthritis and allergies (Sohal, 2002; Squacrito and Peyer, 1998; Choi *et al*., 2002). Undoubtedly, *in vivo* suppression of these reactive species is important for the human body to eliminate the toxicity induced by these reactive species. Now a days, research have been carried out to find powerful and nontoxic antioxidants from natural sources, especially edible or medicinal plants to prevent the above reactive species related disorders in human as well as replace the synthetic compounds which are in use may have carcinogenic activity and harmful to the lungs and liver (Rechner *et al*., 2002).

The plant *Oxalis corniculata* (creeping wood sorrel) also called procumbent yellow sorrel belongs to family oxalidaceae. It is very popular perennial herb and distributed in all over Bangladesh. The leaves of wood sorrel are quite edible with a tangy taste and well known for its medicinal value as a good appetizer (Peterson, 1977). The entire plant is rich in Vitamin-C and leaves possess three major C-glycosyl flavones namely isoorientin, isovitexin and swertisin (Mizokami *et al*., 2008). *Oxalis corniculata* used in wound healing (Taranalli *et al*., 2004), Abortifacient antimplantation (Sharangouda and Patil, 2007). Antibacterial activity (Satish *et al*., 2008) anti fungal activity (Iqbal *et al*., 2001) relaxant activity (Achola *et al*., 1995) and other traditionally used in anaemia, dyspepsia, cancer, piles, dementia, convulsionis (Chetty *et al*., 2008). It is also reported to exhibit hypoglycemic, antihypertensive, antipsychotic, CNS-stimulant and posses chronotropic and inotropic effect (Achola *et al*., 1995; Raghavendra *et al*., 2006).

Recently, Sakat *et al*. (2010) have suggested only *in vitro* antioxidant property of methanolic extract of *Oxalis corniculata*. Taking this in view and as a part of our ongoing search on Bangladeshi medicinal plants (Alam *et al*., 2010) the present study aimed at evaluating the antioxidant potential of the Methanol (MeOH) extract and its organic solvent soluble fractions, such as dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), n-butanol (n-BuOH) and the aqueous (H_2O) fraction, of the *O. corniculata* through various *in vitro* and *in vivo* models. In addition, the toxic potentialities of these fractions were also investigated.

MATERIALS AND METHODS

Plant materials: The whole plant of *Oxalis corniculata* Linn. was collected from the village Kachuria under Mollahat thana of Khulna district, Bangladesh during the month of August 2009. The plants were mounted on paper and the sample was identified by Mrs. Mahmuda Begum, Senior Scientific Officer, Bangladesh National Herbarium, Dhaka, where the voucher specimen has been deposited. Its DACB Accession No. is 32930.

Chemicals: Ammonium molybdate, Folin-chiocaltu phenol reagent, sodium nitroprusside, were purchased from E. Merck (Germany). 1,1-diphenyl- 2-picryl-hydrazyl (DPPH), ascorbic acid, quercetin and potassium ferric cyanide and DL-penicillamine (DL- 2- amino- 3- mercapto- 3- methylbutanoic acid) were purchased from Sigma Chemical Company (St. Louis, MO, USA). The high quality DCFH-DA and DHR 123 (dihydrorhodamine 123) and ONOO- were purchased from Molecular Probes (Eugene, Oregon, USA) and Cayman (Ann Arbor, MI, USA), respectively. All other chemicals and reagents were of analytical grade.

Preparation of plant extract: The plant material was shade-dried with occasional shifting and then powdered with a mechanical grinder, passing through sieve No. 40 and stored in a tight container. The dried powder material (1.5 kg) was refluxed with MeOH for three hours. The total filtrate was concentrated to dryness, *in vacuo* at 40°C to render the MeOH extract (490 g). This extract was suspended in H_2O and then successively partitioned with dicholoromethane (CH_2Cl_2), ethylacetate (EtOAc) and normal butanol (n-BuOH) to afford the CH_2Cl_2 (200 g), EtOAC (60 g), and n-BuOH (110 g) fractions and the H_2O residue (120 g).

***In vitro* antioxidant activity**

The amount of phenolic compounds and flavonoids: The total phenolic and flavonoid content of methanolic extract and several organic fractions were determined using Folin-Ciocalteu reagent (Yu *et al*., 2002) and aluminium chloride colorimetric method (Chang *et al*., 2002), respectively.

Determination of total antioxidant activity: The antioxidant activity of the MeOH extract and several fractions were evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al*. (1999). The assay is based on the reduction of Mo(VI)-Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH. The antioxidant activity is expressed as the number of equivalents of ascorbic acid using the following formula:

$$\text{Antioxident activety} = C = (c \times V)/m$$

where, C is total antioxidant activity in mg g^{-1} plant extract, in Ascorbic acid; c is the concentration of ascorbic acid established from the calibration curve in mg mL^{-1}; V is the volume of extract in mL and m is the weight of pure plant extract in g.

Free radical scavenging activity measured by 1,1-diphenyl-2-picryl-hydrazyl (DPPH): The free radical scavenging activity of MeOH extract and fractions, based on the scavenging activity of the stable 1,1-diphenyl-2- picrylhydrazyl (DPPH) free radical, was determined by the method described by Braca *et al*. (2001). The percentage inhibition activity was calculated from:

$$\% \text{ inhibition} = [(A_0 - A_1)/A_0] \times 100$$

where, A_0 is the absorbance of the control and A_1 is the absorbance of the extract/standard. IC_{50} value was calculated from the equation of line obtained by plotting a graph of concentration (μg mL^{-1}) versus % inhibition.

Measurement of the ONOO-scavenging activity: The ONOO-scavenging activity was measured by monitoring the oxidation of DHR 123, by modifying the method of Kooy *et al*. (1994).

Nitric oxide radical scavenging assay: The procedure is based on the method (Sreejayan and Rao, 1997) where sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions.

Reducing power activity: The reducing power of *O. corniculata* was determined according to the method previously described by Oyaizu (1986).

***In vivo* antioxidant activity**

Animals: Male Wistar rats with a mean weight of 175±5.2 g were collected from the animal research branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR,B). Animals were maintained under standard environmental conditions (temperature: (24.0±1.0°), relative humidity: 55-65% and 12 h light/12 h dark cycle) and had free access to feed and water *ad libitum*. The animals were acclimatized to laboratory condition for one week prior to experiments. All protocols for animal experiment were approved by the institutional animal ethical committee.

Animal grouping and extract administration: Twenty five male rats were randomized into five groups consisting of five each. Group 1 served as normal control and was given distilled water alone (0.5 mL) per day for seven days with the aid of oropharyngeal cannula. Groups 2 animals served as hepatotoxic control, treated with CCl_4 in a single dose of 0.5 mL administered orally for seven days. Groups 3 animals served as positive control, treated with silymarin in a single dose of 25 mg/kg/day orally for seven days while the animals in group 4 and 5 were treated like the normal control except that they received 0.5 mL of the extract corresponding to 250 and 500 mg kg^{-1} body weight respectively. Again group 3-5 was given 0.5 mL of CCl_4 on the seventh day after 6 h of extract administration. All the animals from each group were sacrificed by ether anesthesia 24 h after their respective 21 daily doses of the extract and distilled water. The liver from each animal was excised, rinsed in ice cold 0.25 M sucrose solution and 10% w/v homogenate was prepared in 0.05 M phosphate buffer (pH 7) and centrifuged at 5000 rpm for 60 min at 4°C. The supernatant obtained was used for the estimation of catalase, superoxide dismutase, lipid peroxidation (TBARS) and reduced glutathione.

Determination of catalase activity: The activity of catalase was assayed according the method described by Pari and Latha (2004).

Determination of superoxide dismutase activity: Superoxide dismutase was assayed as described by Naskar *et al.* (2010).

Determination of reduced glutathione activity: Reduced glutathione was determined using the modified method of Ellman (1959).

Estimation of lipid peroxidation: Lipid peroxidation in the liver was estimated colorimetrically by thiobarbituric acid reactive substances (TBARS) using the modification method of Niehaus and Samuelson (1968).

Brine shrimp lethality bioassay: The toxic potentiality of the different fractions of the plant was evaluated using Brine Shrimp lethality bioassay method (Meyer *et al.*, 1982), where 6 graded doses (viz,. 5, 10, 20, 50, 100 and 200 μg mL^{-1}) were used.

Statistical analysis: All values were expressed as the Mean±Standard error of three replicate experiments. The analysis was performed by using student's t test. The $p<0.001$ and <0.005 were considered to be statistically significant.

RESULTS

In vitro antioxidant activity

Total phenolic and flavonoid contents: The content of total phenols in the extract and fractions of *O. corniculata* was determined using the Folin-Ciocalteu assay, calculated from regression equation of calibration curve ($y = 0.013x+0.127$, $r^2 = 0.988$) and is expressed as Gallic Acid Equivalents (GAE). The content of the total phenols in the fractions decreased in the order of EtOAc > n-BuOH > MeOH > CH_2Cl_2 > aqueous fractions and the flavonoid contents of the whole plant extract and fractions in terms of quercetin equivalent (the standard curve eqation: $y = 0.009x-0.036$). The flavonoid content in the fractions decreased in the order of EtOAc > n-BuOH > MeOH > aqueous fractions > CH_2Cl_2 (Table 1).

Total antioxidant activity: Percentage yield of methanol extract and different organic fractions of *O. corniculata* and their total antioxidant capacity are given in Table 1. Total antioxidant capacity of *O. corniculata* is expressed as the number of equivalents of ascorbic acid. Total antioxidant capacity of EtOAc fractions showed the highest and was found to be 224.5±0.45 mg g^{-1} equivalent of ascorbic acid, followed by n-BuOH, MeOH, Aqueous fraction and CH_2Cl_2 125.4±0.21, 113.9±0.69, 20.4±0.12 and 14.0±1.01 mg g^{-1} equivalent of ascorbic acid, respectively.

DPPH radical scavenging activity: All the fractions of *O. corniculata* demonstrated H-donor activity. EtOAc fractions showed the highest DPPH scavenging activity with the IC_{50} value of 4.04±0.08 μg mL^{-1}, followed by n-BuOH, MeOH and aqueous extract/fractions with the IC_{50} value of 12.32±0.16, 17.37±0.22 and 48.49±0.72 μg mL^{-1}, respectively. CH_2Cl_2 had no activity within the experimental concentration range. EtOAc fractions showed the three fold higher activity than the standard ascorbic acid (IC_{50} 12.30±0.11μg mL^{-1}) (Table 2).

Peroxynitrite ($ONOO^-$) scavenging activity: The ONOO-scavenging activity was measured by monitoring the oxidation of DHR 123. The MeOH extract and its organic soluble fractions exhibited significant $ONOO^-$ scavenging effects in a dose-dependent manner, with IC_{50} values of 2.29±0.18 μg mL^{-1} for EtOAc fraction and exerted activity three-fold stronger than a well known $ONOO^-$ scavenger, penicillamine, with an IC_{50} value of 6.20±0.32 μg mL^{-1}. n-BuOH came in second with respect to IC_{50} values, 8.85±0.28, followed by MeOH, aqueous and CH_2Cl_2 extrac/fractions with IC_{50} values of 15.16±0.61, 58.08±2.41 and 82.08±2.41 μg mL^{-1}, respectively (Table 2).

Table 1: Yield, total amount of plant phenolic compounds, flavonoids and total antioxidant capacity of methanolic extract and soluble organic fraction of *Oxalis corniculata*

Sample	Yield (%)	Total phenols mg g^{-1} plant extract (in GAE)[a]	Total flavonoids mg g^{-1} plant extract (in QA)[b]	Total antioxidant capacity mg g^{-1} extract (in ASC)[c]
MeOH	32.66	168.97±0.12	79.98±0.32	113.9±0.69
CH_2Cl_2	13.33	13.85±0.19	1.96±0.41	14.0±1.01
EtOAc	4.00	340.00±0.02	112.73±0.23	224.5±0.45
n-BuOH	7.33	217.38±0.21	97.24±0.12	125.4±0.21
H_2O	8.00	12.77±0.15	10.36±0.02	20.4±0.12

[a]Gallic acid equivalents (GAE, mg g^{-1} of each extract) for the total phenolic content. [b]Quercetin equivalents (mg g^{-1} of each extract) for the total flavonoid content. [c] Ascorbic acid equivalents (mg g^{-1} of each extract) for the total antioxidant capacity. The GAE, QA and ASC values are expressed as Means±SEM of triplicate experiments

Table 2: Antioxidant activities of the *O. corniculata* extract on DPPH, $ONOO^-$ and NO

Sample	[a]DPPH IC_{50} (μg mL^{-1})	[b]$ONOO^-$ IC_{50} (μg mL^{-1})	[c]NO IC_{50} (μg mL^{-1})
MeOH	17.37±0.22*	15.16±0.61*	58.23±0.15*
CH_2Cl_2	>80.0	82.08±2.41*	109.19±0.72*
EtOAc	4.04±0.08*	2.29±0.18**	8.21±0.07**
n-BuOH	12.32±0.16#	8.85±0.28**	15.10±0.33*
H_2O	48.49±0.72*	58.08±2.41*	97.69±0.57*
Ascorbic acid	12.30±0.11		8.22±0.22
Quercetin			14.94±0.12
L-penicillamine		6.20±0.32	

[a]DPPH is the free radical scavenging activity (IC_{50}: μg mL^{-1}). [b]$ONOO^-$ is the inhibitory activity of authentic peroxynitrite (IC_{50}: μg mL^{-1}). [c]NO is the inhibition of NO production (IC_{50}: μg mL^{-1}). *p<0.001 by student's test for values between the sample and the control. **p<0.005 by student's test for values between the sample and the control. # Not significant

Nitric oxide (NO) radical scavenging activity: The MeOH extract and its organic soluble fractions of *O. corniculata* effectively reduced the generation of NO from sodium nitroprusside. As stated in Table 2, The EtOAc fraction showed the highest scavenging activity (IC_{50} values of 8.21±0.07 μg mL^{-1}) which was similar than the standard ascorbic acid (IC_{50} values of 8.22±0.22 μg mL^{-1}). n-BuOH (15.10±0.33 μg mL^{-1}), MeOH (58.23±0.15 μg mL^{-1}), aqueous (97.69±0.57 μg mL^{-1}) and CH_2Cl_2 (109.19±0.72 μg mL^{-1}) also showed good scavenging activity.

Reducing power ability: For the measurement of the reductive ability, transformation of Fe^{3+} to Fe^{2+} was investigated in the presence of extract and organic fractions. Like the antioxidant activity, the reducing power of *O. corniculata* increased with increasing concentration of the sample. Figure 1 shows the reductive capabilities of the *O. corniculata* compared with quercetin. All *O. corniculata* extract and fractions concentrations tested showed higher activities and these differences were statistically significant (p<0.001).

***In vivo* antioxidant activity**

Estimation of lipid peroxidation (LPO), enzymic (CAT, SOD) and non enzymic (GSH) antioxidant system: Reduced activities of enzymic (CAT, SOD), non enzymic (GSH) antioxidant system and lipid peroxidation (LPO) level of liver homogenate were summarized in Table 3. There was a significant decrease in the percentage inhibition of CAT, SOD and GSH in CCl_4 treated rats than the normal control group. However, the percentage inhibition of SOD, CAT and GSH were significantly increased after oral administration of extract/fractions at 250 and 500 mg kg^{-1} body weight in a dose dependent manner. EtOAc fraction at a dose of 500 mg kg^{-1} body weight showed the highest percentage inhibition activity in both enzymic (69.12±0.11% for CAT and 75.22±0.22% for SOD) and non enzymic (70.45±0.53% for GSH) antioxidant system. Since aqueous and CH_2Cl_2 fractions showed lower activity in *in vitro* model, test for *in vivo* model were not done.

In vivo lipid peroxidation study of rats treated with CCl_4 showed a significant increase (p<0.001) in TBARS when compared with normal control group. Treatment with *O. corniculata* extract/fractions for 7 days were able to lower the rise in TBARS level dose dependently as shown in Table 3. EtOAc showed the highest lowering effect in TBARS level (89.17±0.17) at dose of 500 mg kg^{-1} body weight than the standard Salymarin (72.97±0.27) at dose of 25 mg kg^{-1} body weight.

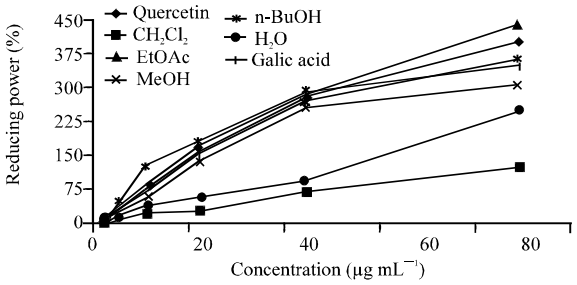

Fig. 1: Reducing power of MeOH extract and fractions of *O. corniculata* and standards by spectrophotometric detection of Fe^{3+} to Fe^{2+} transformation. Results are Mean±SEM of three parallel measurements

Table 3: Effect of MeOH extract and its organic soluble fractions of *O. corniculata* on LPO, antioxidant enzymes and GSH in CCl_4 induced liver damage in male wistar rats

Sample	CAT	SOD	GSH	TBARS
Normal control	91.27±0.19	88.22±0.11	89.55±0.13	95.67±0.17
CCl_4 control	32.12±0.39	42.92±0.21	33.85±0.23	45.17±0.57
Salymarin	75.72±0.19	80.82±0.11	65.95±0.13	72.97±0.27
M1	37.12±0.25*	43.92±0.21*	40.95±1.03*	56.17±0.77*
M2	40.12±0.11*	49.22±0.22*	44.15±0.34*	60.17±0.37*
C1	ND	ND	ND	ND
C2	ND	ND	ND	ND
E1	47.12±0.29*	53.92±0.21*	50.95±0.03*	66.17±0.57*
E2	69.12±0.11*	75.22±0.22*	70.45±0.53*	89.17±0.17*
B1	40.12±0.19*	47.92±0.22*	45.95±0.13*	59.17±0.17*
B2	55.12±0.51*	62.22±0.12*	59.45±0.13*	73.17±0.77*
A1	ND	ND	ND	ND
A2	ND	ND	ND	ND

Values are Mean±SEM, (n = 5). **p<0.001 by student's test for values between the sample and the CCl_4 control. M: Methanolic extract, C: Dicholoromethane (CH_2Cl_2) fraction, E: Ethylacetate (EtOAc) fraction, B: n-Butanol (BuOH) fraction and A: Aqueous (H_2O) fraction. 1: 250 mg kg^{-1} body weight and 2: 500 mg kg^{-1} body weight. ND: Note done

Table 4: LC_{50} data of test samples of *O. corniculata* and vincristine sulphate

Sample	LC_{50} (µg mL^{-1}) Mean±SE[a]
MeOH	85.32±1.63
CH_2Cl_2	29.02±1.16
EtOAc	34.92±1.56
n-BuOH	54.65±3.13
H_2O	> 200
Vincristine sulphate	1.225±0.11

[a]Values of toxicity (LC_{50}) were expressed as the Mean±SE of three experiments

Assay for toxicity of *Oxalic corniculata* extract: As summarized in Table 4, the toxicity exhibited by the crude MeOH extract as well as the organic soluble fractions of the plant showed potent activity against the positive control (vincristine sulphate). The toxicity of the MeOH extract and its fractions on the BSLA increased in the order of CH_2Cl_2 < EtOAc < n-BuOH < MeOH < H_2O and LC_{50} values were 29.02±1.16, 34.92±1.56, 54.65±3.13, 85.32±1.63 and >200 µg mL^{-1}, respectively.

DISCUSSION

Phenolic compounds are commonly found in both edible and nonedible plants, and they have been reported to have multiple biological effects, including antioxidant activity. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Soares *et al*., 1997). Phenolic compounds are understood to induce the cellular antioxidant system; increase approximately 50% cellular glutathione concentration. Flavonoids are important in the modulation of γ-glutamylcysteine synthase in both cellular antioxidant defenses and detoxification of xenobiotics (Muchuweti *et al*., 2007). In this study EtOAc fractions possessed the highest amount of phenolic and flavonoid compounds i.e., 340±0.02 mg g^{-1} in GAE and 112.73±0.23 mg g^{-1} in QA, respectively, followed by n-BuOH and MeOH extract/fractions.

The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the compounds having antioxidant property and the formation of a green phosphate/ Mo (V) complex with a maximal absorption at 695 nm. This assay is successfully used to quantify vitamin E in seeds. This method is simple and independent on other antioxidant measurements and is commonly employed for plant extracts (Prieto *et al*., 1999).

The stable DPPH radical model is widely used and was found relatively quick method for the evaluation of free radical scavenging activity. DPPH• is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Nakayama, 1994). DPPH• is usually used as a substrate to evaluate the antioxidant activity of a compound (Chang *et al*., 2002). Based on the data obtained from this study, DPPH radical scavenging activity of EtOAc fractions of *O. corniculata* was significantly lower than standard. It was revealed that organic soluble fraction of *O. corniculata* did show the proton donating ability and could serve as free radical inhibitor or scavenger, as well as a primary antioxidant that reacts with free radicals, which may limit free radical damage occurring in human body.

Suppression of $NO^{\bullet}$ release may be partially attributed to direct $NO^{\bullet}$ scavenging, at all concentrations of crude methanolic extract and organic fractions of *O. corniculata* which decreased the amount of nitrite generated from the decomposition of sodium nitroprusside *in vitro*. The plant products may have the property to counteract the effect of $NO^{\bullet}$ formation and in turn may be of considerable interest in preventing the ill effects of excessive $NO^{\bullet}$ generation in the human body. Further, the scavenging activity may also help to arrest the chain of reactions initiated by excess generation of $NO^{\bullet}$ that are detrimental to the human health. Nitric oxide is implicated for inflammation, cancer and other pathological conditions (Duh, 1998).

Formation of reactive peroxynitrite ($ONOO^{-}$) form the combination of NO• and $O^{\bullet -}_{2}$ leads to serious toxic reactions with biomolecules such as protein, lipids and nucleic acids. High concentration of Nitric Oxide (NO) has deleterious effects, so it is necessary to regulate the production of NO strictly (Beasley *et al*., 1991). When NO is produced by macrophages, the nitric oxide radical can be converted into peroxynitrites, which will cause diverse chemical reactions in a biological system including nitration of tyrosine residue of protein, triggering lipid peroxidation, inactivation of aconites, inhibition of mitochondrial electron transport and oxidation of biological thiol compound.

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Beckman *et al*., 1990). At the concentration of the extract/fractios tested, reducing power of the extract/fractions was higher than that of some commonly consumed green

leafy vegetable (Oboh, 2008). The reducing properties are generally associated with the presence of reductones which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Beckman and Koppenol, 1996). Present data on the reducing power of the tested extracts suggested that it is likely to contribute significantly towards the observed antioxidant effect.

CCl_4 Is one of the most commonly used hepatotoxin in the experimental study of liver damage (Lee *et al*., 2001). The toxic effects of CCl_4 in vivo is well known to be mediated through radical reactions. The CCl_3O^* and/or CCl_3OO^* redicals produced as a result of the metabolic conversion of CCl_4 is reported to initiate lipid peroxidation (Gupta *et al*., 2006). In the present study, a single dose of CCl_4 developed significant hepatic damage and oxidative stress, leads to increase lipid peroxidantion. The treatment with different fractions of *O. corniculata* was able to reduce the level of lipid peroxides in a dose dependent manner as compared with the hepatotoxic group.

Superoxide dismutase has been reported as one of the most important enzymes in the enzymatic antioxidant defense system (Curtis *et al*., 1972). It removes superoxide anion by converting it to hydrogen peroxide and prevent the toxic effect caused by this radical. CCl_4 Induced hepatic damage lead to decrease in percentage inhibition of SOD and after administration of plant extract/fractions increased the percent inhibition of SOD, revealed the efficient protective mechanism of this plant.

Catalase, another antioxidant enzyme, is widely distributed in the animal tissues and decomposes H_2O_2 and protects the cells from highly reactive hydroxyl radicals (Chance *et al*., 1952). Yeh and Yen (2006), reported that four different phenolic acids induced antioxidant enzymes SOD, catalase and glutathione peroxidase. Thus increased the percentage inhibition of catalase after administration of extract/fractions probably due to the presence of the phenolic compounds in the extract/fractions.

Reduced Glutathione (GSH) is a tripeptide, non enzymatic biological antioxidant present in the liver and are important for maintaining the structural and functional integrity of different organs. Glutathione reductase and NADH stricly maintain the cellular GSH levels (Ganie *et al*., 2010). Moreover, GSH protects cellular proteins against reactive oxygen species generated from exposure to CCl_4 (Arivazhagan *et al*., 2000). The ability of plant extracts to reactivate the hepatic glutathione reductase was reflected by decreasing the level of lipid peroxidation. This result agrees with the earlier report of Bhandarkar and Khan (2004).

The Brine Shrimp Lethality Assay (BSLA) has been used routinely in the primary screening of the crude extracts to assess the toxicity towards brine shrimp, which could also provide an indication of possible toxicity of the test materials. A number of novel antitumor and pesticidal natural products have been isolated using this bioassay (Meyer *et al*., 1982). The variation in BSLA results (Table 4) may be due to the difference in the amount and kind of toxic substances (e.g., tannins, flavonoids, triterpenoids, or coumarins) present in the crude extracts. Moreover, this significant lethality of the crude plant extracts (LC_{50} values less than 100 ppm or $\mu g\ mL^{-1}$) to brine shrimp is indicative of the presence of potent toxic and probably insecticidal compounds which warrants further investigation. BSLA results may be used to guide the researchers on which crude plant extracts/fractions to prioritize for further fractionation and isolation of these bioactive compounds.

CONCLUSION

In conclusion, the results of the present study indicate that the MeOH extract and its various fractions extract exhibit interesting antioxidant properties via various *in vitro* and *in vivo* model

and also show potent toxicity. These results of the investigation do not reveal that which chemical compound is responsible for aforementioned activity. To explore the lead compounds liable for aforementioned activity from this plant are in progress.

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