International Journal of Agriculture, Environment & Biotechnology
Citation: IJAEB: 7(3): 441-454 September 2014
DOI Number: 10.5958/2230-732X.2014.01348.5

Genetics and Plant Breeding

An *in vitro* and *ex vivo* Study on Antioxidant Activity of *Coriander* Seeds

Dasmeet Kaur*[1], Vishal Chugh[1,2] and Giridhar Soni[1]

[1]Department of Biochemistry, Punjab Agricultural University, Ludhiana, Punjab, INDIA.
[2]Akal School of Biotechnology, Eternal University, Baru Sahib, Himachal Pradesh, INDIA.

Corresponding author: dasmeet.kaur@gmail.com

Paper No. 229 Received: December 8, 2013 Accepted: February 17, 2014 Published: August 18, 2014

Abstract

The antioxidant activity of extracts of different polarity from seeds of Local variety of Coriandrum sativum was assessed via both in vitro and ex vivo models. Results indicate that hexane extract of coriander exhibited no appreciable affects at either of the concentrations in all the three in vitro free radical scavenging models i.e. hydroxyl radical, super oxide radical and nitric oxide radical. Instead, it showed pro-oxidant activity at higher concentrations. Whereas methanol: hexane extracts of coriander were found to be ineffective in quenching hydroxyl radical but revealed only moderate activity in quenching super oxide radical (EC_{50}=711.81 µg/ml) and nitric oxide radical (EC_{50}=569.02 µg/ml). Only methanolic extract of Local variety offered strong antioxidant activity in a concentration dependent manner for the same three free radicals with EC_{50} of 18.33 µg/ml, 553.50 µg/ml and 179.53 µg/ml respectively. Even in vitro linoleic acid peroxidation model proved methanolic extract of coriander (80% inhibition till 48 h) to be far better than standard antioxidant ascorbic acid (70% inhibition till 12 h). EC_{50} and SC_{50} for ex vivo linoleic acid peroxidation of rat liver homogenate and erythrocytes were recorded as 383.10 µg/ml and 166.78 µg/ml respectively. The methanolic extract of Local variety was further evaluated for its protective effect on osmotic fragility and antioxidant enzymes of erythrocytes at various time intervals (24-96 h). The decrease in% hemolysis and protection of activities of antioxidant enzymes even for 96 h of incubation in test groups supplemented with coriander proved indubitably, the effectiveness of methanolic coriander extract in alleviating protective mechanisms.

Highlights

- Methanolic extract of *Coriander sativum* offered strong antioxidant activity.
- Coriander protects the erythrocytes integrity as well as increased the resistance of the cells to loss in the activity of their antioxidant enzymes *in vitro*.

Keywords: Coriandrum sativum, Free radicals, Oxidative stress, in vitro and ex vivo antioxidative potential

Reactive oxygen species (ROS), such as hydroxyl radicals and hydrogen peroxide (H_2O_2), super oxide anion ($O_2^{\cdot-}$), nitrogen derived free radicals are nitric oxide ($NO^{\cdot}$) and peroxynitrite anion ($ONOO^{-}$) are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism (Fang *et al.,* 2002). They are constantly generated as normal by-products of mitochondrial respiration and other metabolic activities (Manavalan and Ramasamy, 2001). An imbalance between the generation of ROS and cellular antioxidant

capacity can result in cell membrane disintegration by reacting with membrane proteins or lipids leading to oxidative stress. Moreover ROS can cause DNA mutation which further initiate or propagate development of many diseases (Valentao *et al.,* 2002). Among the pathologies linked to oxidative stress are atherosclerosis, carcinogenesis, aging, physical injury, infection, asthma, cardio vascular malfunction, stroke, vasospasms, liver damage, hypertension, diabetes, inflammation, Parkinson's and Alzheimer's diseases (Benz and Yao, 2008). In treatments of these diseases, antioxidant therapy has gained an immense importance as antioxidants have been reported to prevent oxidative damage by free radical and ROS; any may prevent the occurrence of diseases, cancer and aging (Ray and Husain, 2002). It can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals, and also by acting as oxygen scavengers.

In recent years, plants have played a significant role in providing the human race with remedies. At present, phytotherapy is a recognized complementary and alternative medicinal (CAM) therapeutic modality (DeSylvia *et al.,* 2011). Therefore, there is an increasing interest in natural food additives, such as spices or spice extracts, which can function as natural antioxidants besides seasoning the food.

Coriandrum sativum L. (CS), commonly known as "Coriander" is an umbelliferous annual small plant like parsley which dates back to around 1550 BC, and is one of the oldest spice crops in the world. Although, it is probably native to the eastern Mediterranean and Southern Europe, but India is the largest producer of coriander as the finely ground seeds are used extensively in curry powder according to Indian dietary pattern. Different parts of the plant, including the fruits and the green herbs, are used for medicinal purposes such as alleviate spasms, gastric complaints, bronchitis, gout, recovering loss of appetite and giddiness (Sreelatha *et al.,* 2009). Also, the previous studies on this herb have shown its antidiabetic, antioxidant, hypocholesterolemic, antihelmintic, antibacterial, hepatoprotective, anticancer, anxiolytic (Asgarpanah *et al.,* 2012) and anti-obesity (Dwivedi and Kumar, 2012) activities. The phenolic compounds, apigenin, catechin and p-coumaric acid, and aliphatic alkenals and alkanals were reported in C. sativum aerial parts (Oganesyan *et al.,* 2007) while linalool, geranyl acetate and petroselinic acid were found in the fruit (Momin *et al.,* 2012). The phenolic compounds, caffeic acid derivates, flavonoids and terpenoids are suggested to be responsible for antioxidative effect (Madsen and Bertelsen, 1995). Hence reckoning the immense importance of natural antioxidants in the reduction of free radicals and oxidized compounds, efforts to gain extensive knowledge regarding power of antioxidants from various extracts of Coriandrum sativum, is therefore an area of interest.

Previous preliminary phytochemical investigations have indicated the antioxidant potential of coriander in different in vitro models. However, the antioxidant potential is highly variable with respect to the variety, cultivation practices and location (Dragland *et al.,* 2003). Several analytical methods have been developed to determine the antioxidant capacity of natural substances in vitro but reports on ex vivo models are scanty and limited. The in vitro models can be categorized into two groups: (i) assays for radical-scavenging ability and (ii) assays for lipid oxidation inhibitory effect. However, the total antioxidant activities of plant extracts cannot be evaluated by using one single method, due to the complex composition of phytochemicals as well as of oxidative processes. Therefore, the use of at least two methods should be employed in order to evaluate the total antioxidant activity (Bohm *et al.,* 2001). Since, the results of the said in vitro models depend on a large number of factors; there was a need to have a model system more close to an in vivo system. Consequently, for more extensive and closer study related to human body, an ex vivo model like lipid peroxidation in rat liver homogenate and erythrocyte haemolysis in human beings were studied. The aim of present study was to investigate the antioxidant activity of extracts of different polarity from seeds of Local variety of coriander via both in vitro and ex vivo models.

Materials and Methods

Local variety of Coriandrum sativum was obtained from Department of Agronomy and Agro meteorology, Punjab Agricultural University, Ludhiana. About 100 g

of shade dried coriander seeds were ground to a fine powder and were exhaustively extracted with solvents of varying polarities such as hexane, methanol and hexane-methanol mixture (3:7) by refluxing at 60°C for 12 hours. After filtration, the residues were re-extracted twice under same conditions. Solvent was removed from the combined filtrates under vacuum at 45°C in Buchi rotary evaporator. The yield of crude extracts was determined gravimetrically. These extracts were then stored in a desiccators until further use. The crude extracts were re-dissolved in distilled water when necessary for assessment of antioxidant activity. For the evaluation of antioxidant potential, the extracts obtained from solvents of varying polarity were tested in different in vitro models (hydroxyl radical, superoxide radical and nitric oxide radical scavenging potential, lipid peroxidation using linoleic acid emulsion) and ex vivo models (lipid per oxidation in rat liver homogenate and erythrocyte haemolysis in human beings).

Determination of hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was determined by deoxyribose degradation method (Halliwell *et al.,* 1987) on the basis of study of competition between deoxyribose and test compounds for hydroxyl radicals produced by Fe^{2+}-ascorbate-EDTA-H_2O_2 system (Fenton reaction). The hydroxyl radicals attack deoxyribose that eventually results in formation of thiobarbituric acid reacting substances (TBARS) method (Ohkawa *et al.,* 1979). The reaction mixture consisted of 0.1 ml of deoxyribose (2.8 mM), 0.1 ml of $FeCl_3$ (0.1 mM), 0.1 ml of EDTA (0.1 mM), 0.1 ml of ascorbic acid (0.1 mM), 0.1 ml of H_2O_2 (1 mM), phosphate buffer (20 mM, pH 7.4) and various concentrations of coriander extracts in a final volume of 1 ml. The% inhibition was determined by comparing the absorbance values at 532 nm of test and control.

Determination of superoxide radical scavenging activity

Superoxide scavenging activity was determined by inhibition of light induced (photoreduction) superoxide radical generation by riboflavin and subsequent reduction of Nitroblue tetrazolium (NBT) (Mc-Cord *et al.,* 1969). The reaction mixture consisted of 0.1 ml of EDTA (6.6 mM) containing 3 µg NaCN, 0.1 ml of riboflavin (2 µM), 0.1 ml of NBT (50 µM), various concentrations of coriander extracts and phosphate buffer in a final volume of 3 ml. The% inhibition of superoxide generation was measured at 530 nm by comparing the absorbance values of test and control compounds.

Determination of nitric oxide radical scavenging activity

Nitric oxide radical scavenging was determined by detecting the inhibition of nitric oxide generation as per the method of Green *et al.,* (1982). Reaction mixture consisted of 1ml of sodium nitroprusside (10 mM), various concentrations of coriander extracts and phosphate buffered saline (PBS) to make a total volume of 3 ml. The% inhibition of nitric oxide generation was measured at 546 nm by comparing the absorbance value of test and control.

Determination of lipid peroxidation inhibiting activity using linoleic acid emulsion

The antioxidant activity of coriander was determined by detecting the ability of extract to inhibit H_2O_2 induced lipid peroxidation of linoleic acid. The degree of oxidation was measured according to the thiocyanate method (Mitsuda *et al.,* 1969). Vitamin C was used as a positive control.

In vivo lipid peroxidation of rat liver homogenate

Antioxidant potential of coriander was also evaluated by their ability to prevent lipid peroxidation (LPO) induced by Fe^{2+}-ascorbate system in rat liver homogenate (Bishayee *et al.,* 1970). The% inhibition of lipid peroxidation by the extract was determined by comparing the absorbance values read at 532 nm of control and experimental tubes.

Preparation of packed cell volume and erythrocyte lysate

Human blood sample from healthy volunteers was collected in EDTA containing vials and centrifuged at 1000 x g for 10 min at 4°C to isolate erythrocytes for

biochemical analyses. The buffy coat of erythrocyte pellet was washed three times with phosphate buffered saline (PBS, pH 7.4) to obtain a constantly packed cell volume (PCV) preparation. After washing, PCV was adjusted to 5% with 0.9% saline solution for determining LPO. To prepare erythrocyte lysate, 0.8 ml of distilled water was added to 0.2 ml of 5% PCV. The lysed cell preparation was used for the estimation of antioxidant enzymes.

Determination of osmotic fragility of erythrocytes

Osmotic fragility of erythrocytes was estimated by determining hemolysis of erythrocytes in different concentrations of NaCl solution (Dacie and Lewis 1968). Saline concentrations were divided into 10 grades, varying from 0.1% to 0.9% and 50 µl of blood was added to 5 ml of each of these solutions containing different concentrations of saline. The solutions were incubated at 37°C for 30 min and centrifuged. The absorbance of supernatant was recorded at 490 nm using supernatant from the blood suspension in 0.9% saline as blank. The percent hemolysis in different saline solutions was calculated assuming the hemolysis in water as 100%. Percent hemolysis in different concentrations of sodium chloride was plotted against saline concentration and the concentration of saline needed for 50% hemolysis was determined.

Determination of LPO in erythrocytes

The LPO in erythrocytes was determined by measuring the malondialdehyde (MDA) produced using thiobarbituric acid (TBA) by the method of Stocks and Dormandy, (1971). The percent degree of LPO was expressed as nmoles of MDA produced, using a molar extinction coefficient of pure MDA as 1.56 x 105 (Esterbauer *et al.,* 1982). The percent protection was calculated by percent decrease in LPO of erythrocytes in the presence of various concentrations of coriander extracts.

Determination of protein content

Protein was estimated by the method of Lowry *et al.,* (1951) using Folin phenol reagent. A calibration curve was prepared using crystalline bovine serum albumin (50-500 µg) as the standard.

Estimation of Antioxidant enzymes

The activity of catalase (EC 1.11.1.6) in erythrocyte lysate was determined according to the method described by Aebi, (1983). The decomposition of H_2O_2 can be followed directly by recording decrease in absorbance at 240 nm. The results were expressed as µmoles of H_2O_2 decomposed/min/mg protein using 36 as molar extinction coefficient of H_2O_2.

Superoxide dismutase (SOD) (EC 1.15.1.1) activity was determined by the method of Marklund and Marklund, (1974). The assay of SOD is based upon the ability of this enzyme to inhibit the auto-oxidation of pyrogallol in the presence of EDTA. A unit of enzyme activity is defined as the amount of the enzyme causing 50% inhibition of the auto-oxidation of pyrogallol observed in the control.

The activity of peroxidase (EC 1.11.1.7) was determined by the method of Claiborne and Fridovic, (1979). The results are expressed as µmoles of o-dianisidine oxidized/ min/mg protein using 8.3 x 103 L mol^{-1} cm^{-1} as the molar extinction coefficient.

Results and Discussion

Yields of the extracts of *Coriandrum sativum* with different solvents were quite comparable. Local variety of coriander exhibited 6.65%, 6.27% and 6.61% yield in case of methanolic, methanol:hexane and hexane extracts respectively.

Hydroxyl radical scavenging activity

Among the several free radicals, hydroxyl radical ($OH^{\bullet}$) is the most potent oxidant, produced during radiation exposure as well as Fenton reaction (Naik *et al.,* 2006). No appreciable effect of hexane as well as methanol:hexane extracts was seen at either of the concentrations in this model. Instead, the extracts showed pro-oxidant activity at higher concentrations, thereby ruling out the free radical scavenging potential of both the extracts (Fig. 1a, b). However, the methanolic extract of coriander was highly effective in scavenging hydroxyl free radicals in a concentration dependent manner (Fig.1c). Concentrations of local variety needed for 50% inhibition of hydroxyl radical generation was found to be 18.33 µg/ml (Table 3). EC_{50} equivalents/g of coriander for hydroxyl radical scavenging potential was found to be 3628.

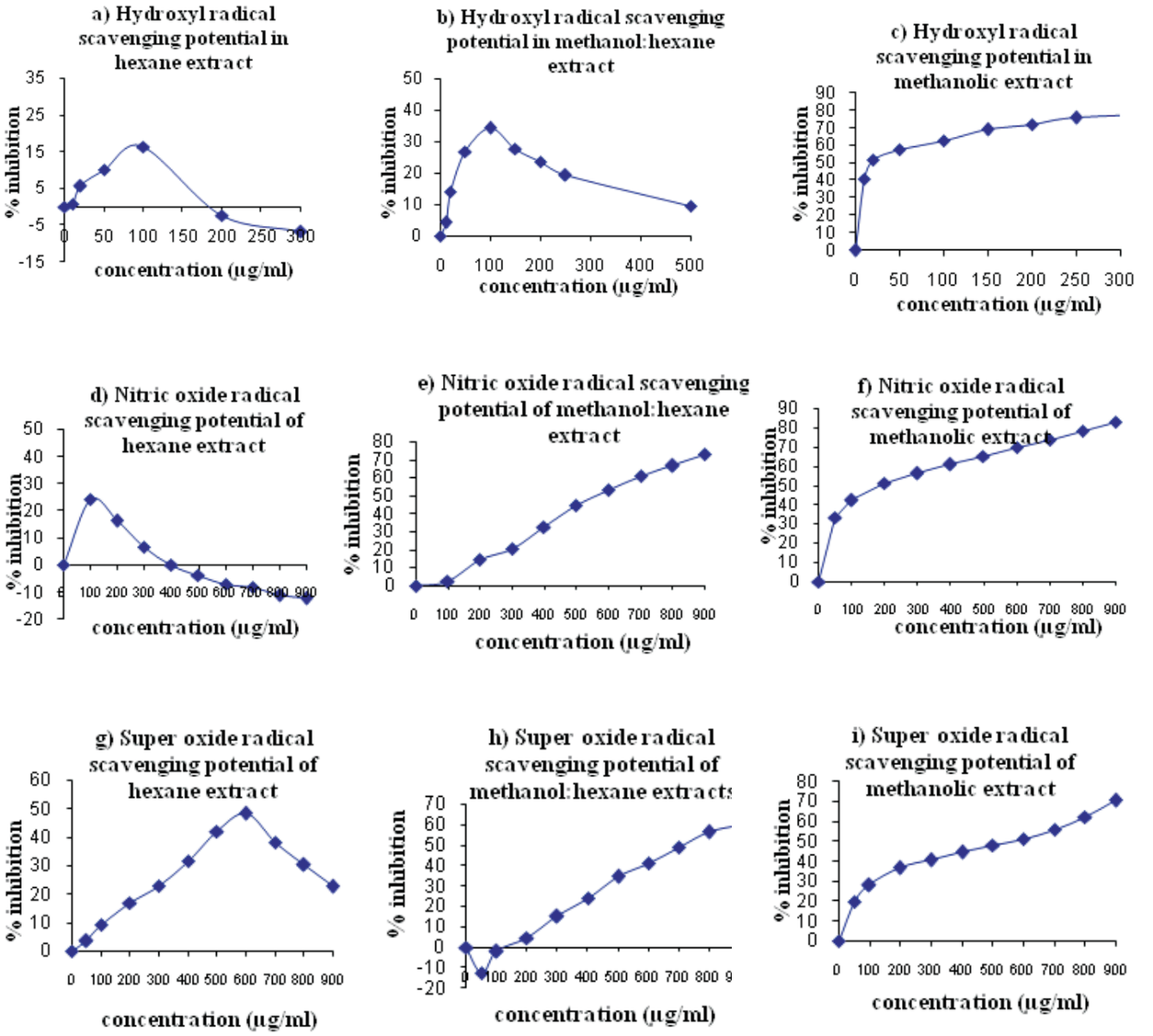

Fig. 1 Effect of various extracts of Local variety of coriander on oxygen radical generation in vitro- (a,b,c) hydroxyl radical (d,e,f) superoxide radical (g,h,i) nitric oxide radical.

Superoxide radical scavenging activity

Superoxide anion is the first reduction product of oxygen which is measured in terms of inhibition of generation of $O_2^{\bullet -}$. Hexane extracts were found to be ineffective in inhibiting the formation of superoxide radicals at either of the concentrations. Instead, the extract showed pro-oxidant behavior at high concentrations, thereby, proving hexane extract to be ineffective towards superoxide radical scavenging assays (Fig. 1d). However, the methanol:hexane extract of local variety showed reasonable antioxidant activity against superoxide radical generation (Fig. 1e). The result clearly depicted that methanol:hexane extract scavenged the superoxide radicals generated by photo-

reduction of riboflavin in a concentration dependent manner to a good extent. The concentration of Local variety of coriander needed for 50% inhibition (EC_{50}) was found to be 711.8 µg/ml (Table 3) with EC_{50} equivalents/g of coriander of 88.1equivalents).Whereas the methanolic extracts of coriander exhibited even better inhibition against superoxide radicals generated by photo reduction of riboflavin in concentration dependent manner where methanolic extract of Local variety displayed the better potential than methanol:hexane extract (Fig 1f) and concentration needed for 50% inhibition (EC_{50}) was found to be 553.50 (Table 3) with EC_{50} equivalents/g of coriander computed to be 120.14.

Once again the results are in favor of methanolic extracts and it can be concluded that it was only methanolic extract that exhibited the highest antioxidant activity as compared to the activity of extracts in other solvents. This observation is supported by the reports of Chang *et al.,* (1977) who reported methanol to be effective solvent for extraction of antioxidants from rosemary and sage. The methanolic leaf extracts of *Piper sarmentosum* (Kodak) and *Morindaelliptica* (Mengkudu) are also reported to possess high superoxide radical scavenging activity (Subramaniam *et al.,* 2003). However, Satyanarayana *et al.,* (2004) reported that aqueous extracts of coriander inhibited superoxide anion in *in vitro* system and 50% scavenging of superoxide radical occurred at concentration of 370 µg/ml of coriander.

Nitric oxide radical scavenging activity

The nitric oxide radical production by the incubation of solution of sodium nitroprusside in standard phosphate buffer at 25°C was not reduced by hexane extracts of coriander. With increasing concentrations rather a pro-oxidant activity became dominant in the assay system, thus, ruling out the use of hexane extracts for isolation of antioxidants from the coriander seeds (Fig 1g). Inhibition of nitric oxide radical generation by methanol:hexane extracts of coriander was however quite appreciable (Fig 1h). The nitrite production in *in vitro* assay was lessened by these extracts and the concentrations of Local variety needed for 50% inhibition and EC_{50} equivalents/g were determined to be 569.0 µg/ml and 110.2 respectively (Table 3). Inhibition potential of methanolic extract of coriander against nitric oxide radical generation proved better than that of hexane and methanol:hexane extracts with EC_{50} value of 179.53 µg/ml and the EC_{50} equivalents/g of coriander were calculated to be 370.41 (Fig. 1i). This highlights the better antioxidative potential of methanolic extract of Local variety of coriander. The inhibition may be due to the antioxidant principles in the extract which compete with oxygen to react with nitric oxide thereby inhibiting the generation of nitrite (Marcocci *et al.,* 1994). The concentration of ascorbic acid needed for 50% inhibition was found to be much higher i.e. 2700 µg/ml.

Nitric oxide reacts with other radicals such as $O_2^{\bullet -}$ to form peroxynitrite ($ONOO^-$), a powerful oxidant and this reaction is widely believed to represent a major pathway for generating reactive nitrogen species *in vivo* (Leeuwenburgh and Heinecke, 2001). Hence, the excess production of NO can induce oxidation of proteins, lipids and DNA and is associated with several diseases (Hamilton *et al.,* 2004).To avoid or delay this peroxidation process, addition of antioxidants to foods is the most extensive method. Rekha *et al.,* (2001) assessed the antioxidant activity of brahma rasayana by its ability to scavenge the hydroxyl generated by Fenton reaction, superoxide radical generated by photoreduction of riboflavin, nitric oxide radical generated from sodium nitroprusside and found the concentrations needed for their 50% inhibition to be 7400, 180 and 4 µg/ml respectively.

The potency of added extract also depends on the presence of numerous micro-components acting as pro-oxidant or synergists (Kamal Eldin and Appleqvist, 1996). The concentration needed for 50% inhibition of generation of radicals varied due to difference in the sensitivity of each assay. Thus, methanolic extracts of coriander resulted out to be effective scavenger of hydroxyl radical (HO˙), superoxide radical (O2˙-) and nitric oxide radical (NO˙). As reported earlier also, methanolic extracts exhibited most potent in vitro antioxidant activity with high percentage inhibition as compared to other solvents (Masada *et al.,* 2013). Hexane extracts showed weak antioxidant activity, rather they showed pro-oxidant activity at higher concentrations. These observations support the earlier reports of Melo *et al.,* (2003) who established the fact that hexane extracts of coriander

testified weak antioxidant activity, rather they showed pro-oxidant activity at higher concentrations. Since only the methanolic extracts were effective anti-oxidants it appears that the antioxidant potential of coriander could be attributed to its high flavonoid content. So from the above free radical scavenging assay observations, we concentrated on the methanolic extracts of Local variety for further study.

Inhibition of linoleic acid peroxidation

Lipid peroxidation, caused by free radicals leading to oxidative destruction of PUFA (Polyunsaturated fatty acids) a constituent of cellular membranes produces toxic and reactive aldehyde metabolite i.e. malondialdehyde (MDA) which is most commonly measured as TBARS. The methanolic extract of coriander exhibited inhibition of the peroxidation of linoleic acid in a concentration dependent manner (Table 1). The coriander extract was highly effective in preventing the peroxidation of linoleic acid even up to 48 h at higher concentrations.

This observed activity of coriander extract was even more effective than the positive control (ascorbic acid) used in the study which gained the maximum inhibition of 75% till 12h where as coriander exhibited maximum inhibition of 80% till 48h of incubation period. The functional methods that simulate oxidative reactions similar to those occurring *in vivo* explore their protective effects against oxidative reactions (Kaur and Kapoor, 2002, Lavellei *et al.,* 2000). As relatively polar solvent extract showed better antioxidant property the presence of various flavonoids in herbal extracts might have been involved in inhibition of peroxidation.

Inhibition of lipid peroxidation in rat liver homogenate

Addition of herbal methanolic extract of local variety of coriander at various concentrations was found to inhibit peroxides generated by Fe^{2+}-ADP-ascorbate in rat liver homogenate in a dose dependent manner (Table 2). The concentration of local variety of coriander needed for 50% inhibition was found to be 383µg/ml. The protection of membrane lipids by the methanolic extracts is evidenced by the MDA formed and% protection in *in vitro* lipid peroxidation induced in rat liver homogenate. Rekha *et al*., (2001) reported that the addition of aqueous extract of brahma rasayana prevent the lipid peroxidation of rat liver homogenate with an IC50 value of 700µg/ml.

Results of present study reveal that the antioxidant activity of coriander depended on several factors which include presence of the compounds with antioxidant or pro-oxidant activity, methods of extraction, concentration of extract used and finally the method of choice for

Table 1. Antioxidant activity of methanolic extract of coriander (local variety) -%Inhibition of linoleic acid peroxidation

Concentration	Time (h)									
(µg/ml)	0.5	12	24	36	48	60	72	84	96	108
Coriander Local variety (*Coriandrumsativum*)										
20	0.00	38.08	27.61	23.55	18.41	6.67	6.37	0.00	0.00	0.00
50	0.00	44.75	27.61	37.00	27.22	20.37	6.37	1.57	0.00	0.00
100	10.71	61.87	54.47	49.61	40.85	31.62	19.94	12.46	3.13	1.47
150	7.142	66.64	70.26	66.40	61.64	52.66	40.78	32.48	24.01	16.18
200	7.142	72.37	78.64	78.18	80.01	66.46	52.65	45.53	36.29	27.94
Ascorbic acid(*Positive control*)										
100	10.00	32.14	12.10	10.42	1.96	6.62	0.00	0.00	0.00	0.00
200	20.00	35.71	36.59	20.83	11.76	25.74	4.96	2.78	0.00	0.00
500	40.00	50.00	43.90	27.08	17.65	30.88	25.00	12.50	6.94	0.00
1000	55.00	75.00	63.41	37.50	29.41	50.00	30.50	18.06	11.11	0.00

activity test. Al-Mamary, (2002) assayed 17 commonly consumed vegetables for their in vitro inhibition of rat liver homogenate peroxidation and proved coriander possessing exceptionally high antioxidant activity (60%) with 50µl of its juice extracted among all the vegetables. Significant high antioxidant efficiency of coriander, tomato and other vegetables could be attributed to quality and not the quantity of the total efficient phenolics which make them more reactive to neutralize free radicals (Al-Mamary, 2002). The quantity of aqueous extracts of umbelliferous fruits including coriander required for 50% inhibition of lipid peroxidation in in vitro study using rat liver homogenate was found to be < 4600 µg while ascorbic acid requiring 5000 µg, thus proving their effect superior to ascorbic acid (Satyanarayana *et al.,* 2004) which further supports observations of the present investigation.

Inhibition of in vitro lipid peroxidation of erythrocytes

Erythrocytes are preferred extensively to be as a model for investigating oxidant stress since they are vulnerable to oxidative damage as a result of free radical attack, as they are exposed to molecular oxygen directly, rich in metal ions and PUFA which may easily get oxidized and moreover do not have ability to repair membrane damages and regenerate (Etlik and Tomur, 2006). The most commonly used indicator of LPO is TBARS. Increased LPO of erythrocytes could be due to generation of oxygen free radicals (Baneerjee *et al.,* 1999), which in turn may also lead to increased osmotic fragility of erythrocytes.

The data presented in Table 2 establishes that MDA production decreased with increasing concentration of coriander extract and the% protection against lipid peroxidation increased in a dose dependent manner. The EC50 value required for 50% protection was found to be 166.78 µg/ml. The observed in vitro lipid peroxidation of erythrocytes was significantly lower in test as compared to control. Incubation of RBC outside the body at lower temperature produces oxidative stress leading to an increased LPO in both test and control, the increase being relatively lesser in test. Tremendous increase in TBARS suggests an increase in oxygen radicals that could be either due to their increased production and/or decreased destruction (Matkovics *et al.,* 1998).

Dariyerli *et al.,* (2004) suggested the in vivo hemolytic increment value as compared to control group in situation of oxidative stress, maximum hemolysis ratio was the proof of increased osmotic fragility of the erythrocytes. Moreover lipid peroxidation which is an autocatalytic free radical process has been reported to have been inhibited by green tea polyphenols like epigallocatechingallate (EGCG) that can act as an antioxidant by trapping proxyl radicals using erythrocyte membrane-bound ATPases as a model and have been proposed as a cancer chemopreventive (Saffari and Sadrzadeh, 2004). Several constituents in these herbal extract may have counteracted the free radicals through effective scavenging and

Table 2. Antioxidant activity of methanolic extract of coriander (local variety) –Protection of *in vivo* lipid peroxidation in rat liver homogenate and erythrocytes

Concentration (µg/ml)	MDA formed (nmoles)/h/ml of tissue homogenate	% Protection	MDA formed (nmoles)/ ml of PCV	% Protection
20	55.244 ± 2.03	5.99 ± 2.57	7.78 ± 0.49	7.60 ± 1.36
50	51.667 ± 3.69	12.23 ± 3.07	6.57 ± 0.60	21.99 ± 3.98
100	43.321 ± 3.69	26.48 ± 2.83	5.29 ± 0.52	37.21 ± 4.12
200	33.782 ± 2.03	42.57 ± 1.84	3.61 ± 0.71	57.42 ± 6.54
500	27.025 ± 1.49	54.04 ± 1.55	2.56 ± 0.41	69.70 ± 2.90
1000	19.474 ± 1.12	65.57 ± 2.52	1.92 ± 0.39	77.32 ± 3.38
Control	58.821 ± 2.98	-	8.41 ± 0.51	-

Values are mean ± S.D, n = 3

decreased MDA formation. Inhibition of auto-oxidation of PUFA observed ex vivo in the present study is indicative of efficient protective mechanism enhanced by additional antioxidants.

Flavonoids are very effective scavengers of peroxyl radicals and they are also chelators of metals and inhibit the Fenton and Haber - Weiss reactions, which are important sources of oxygen free radicals (Siddhuraju and Becker, 2003). Flavonoids such as isoquercitin and rutin known to be present in coriander may be responsible for antioxidant activity of methanolic extracts (Kunzemann and Hermann, 1977). Among the plant materials, fruits, vegetables and spices are reported to be rich in flavonoids. Thus, the observed antioxidant activity of coriander may be due to the presence of flavonoids in them.

Protection of erythrocyte membrane integrity and antioxidant enzymes

Free oxygen radicals has been reported to induce changes in erythrocytes that results in severe deterioration of membrane structure and function causing increased osmotic fragility, accelerated cell aging and premature cell death ultimately (Etlik and Tomur, 2006). Susceptibility of erythrocytes to auto-oxidation pertaining to high oxidative stress in therapeutic situations or in alternate environments has been accepted as a useful and convenient cell system via ex vivo incubation of RBC and to evaluate the effects of augmented antioxidative potential through supplementation of herbal extracts.

Thus, in order to determine the antioxidant potential of extracts, the human erythrocytes (5%) were stored

Table 3. EC_{50} equivalents of coriander (local variety) extracts with various solvents in different molels

Model used / Solvent used	EC_{50} equivalents*				
	***In vitro* Hydroxyl radical scavenging potential**	***In vitro* Superoxide radical scavenging potential**	***In vitro* Nitric oxide radical scavenging potential**	***Ex vivo* Linoleic acid peroxidation of rat liver homogenate**	***Ex vivo* lipid peroxidation of erythrocytes**
Methanol	18.33 ± 3.86	553.50 ± 20.40	179.53 ± 28.11	383.10 ± 40.40	166.78 ± 25.13
Hexane	-	-	-		
Methanol:Hexane	-	711.81 ± 8.62	569.02 ± 31.18		

Values are mean ± S.D., n=3
*1 EC_{50} equivalent = µg of extract (Effective concentration) required for 50% inhibition/protection of oxidation reaction in a volume of 1ml.

Table 4. Protective role of methanolic extract of coriander (local variety) - Saline concentrations needed for 50% hemolysis of erythrocytes (SC_{50}) and hemolysis of erythrocytes in 0.5% saline

Incubation period (h)	SC_{50} value (%)				% Hemolysis	
	Control	Test Concentrations		Control	Test Concentrations	
		100 µg/ml	500 µg/ml		100 µg/ml	500 µg/ml
24	0.49 ± 0.01	0.46 ± 0.01	0.43 ± 0.01	46.98 ± 2.29	41.54 ± 1.51	36.77 ± 1.41
48	0.53 ± 0.01	0.50 ± 0.01	0.47 ± 0.01	62.71 ± 2.39	50.32 ± 1.32	41.45 ± 1.06
72	0.57 ± 0.02	0.53 ± 0.02	0.48 ± 0.01	70.83 ± 3.40	58.45 ± 1.42	47.63 ± 0.98

Values are mean ± S.D, n=3
SC_{50} at 0 h was found to be 0.42 ± 0.01

with different concentrations of coriander extract for different time intervals at low temperature (4˚C). The osmotic fragility and activities of antioxidant enzymes of erythrocytes were determined from the stored cells. The erythrocyte samples were drawn at different intervals from 24-72 h for osmotic fragility of membranes and 24-96 h to study the effect on antioxidant enzymes of erythrocytes. And to analyze the effect of coriander extract on antioxidant enzymes of erythrocytes, cell lysate was prepared from PCV drawn at various intervals of time.

Effect on osmotic fragility of erythrocytes

For the determination of osmotic fragility, erythrocyte samples were subjected to osmotic shock with varying concentrations of saline. Percent hemolysis was plotted against saline concentration. From the plots, the concentration of saline needed for 50% hemolysis was determined. Results presented in the table 4 demonstrates the osmotic fragility in terms of% hemolysis of erythrocytes stored in the absence and the presence of extracts of coriander at the concentrations of 100 and 500µg for 24 to 72h. For the present analysis, the% hemolysis of erythrocytes in 0.9% saline and 0% saline were assumed to be 0% and 100% respectively. At 0 h, 50% hemolysis (SC_{50}) occurred at 0.42% of saline both in control and the test groups. Moreover, with the increase in storage period, percent hemolysis also increased in its magnitude in both test as well as in control groups but the increase recorded was of lower degree in test groups. After storage period of 24 h, the effective concentrations of saline (SC_{50} values) for test groups with coriander extract (100 and 500 µg/ml) were lower (0.46, 0.43% respectively) than that for control group (0.49%) (Table 4). After 48 h of storage, the respective SC_{50} values jumped to 0.50, 0.47% as compared to the control (0.53%). Even after 72 h of storage, SC_{50} values of erythrocytes in tests (occurred respectively at lower saline concentrations (0.0.53 at 100µg/ml and 0.48% at 500 µg/ml) as compared to that in control (0.57%). It is evident that percent hemolysis increased with increasing storage period and were relatively lower in test groups as compared to the control (Table 4). The assessed percent hemolysis of control in 0.5% saline at 24, 48 and 72 h were 46.98, 62.71 and 70.83 respectively. The percent hemolysis in 0.5% saline after storage for 24, 48 and 72 h in the presence of 100µg/ml of coriander extract were 41.54, 50.32 and 58.45 respectively whereas the percent hemolysis in erythrocytes stored in the presence of 500µg/ml of extract were 36.77, 41.45 and 47.63 respectively. These results support the effectiveness of coriander extract in protecting the integrity of erythrocytes and thus establishing protectiveness of coriander extract towards osmotic fragility. Preincubation of erythrocytes with Nigella sativa and Allium sativum has also been reported to protect erythrocytes against protein degradation, loss of deformability and increased osmotic fragility caused by H_2O_2 (Suboh *et al.,* 2002). These results also support the in vivo observations in which ascorbic acid supplementation has been reported to increase the resistance of the cells to lysis, packed cell volume and hemoglobin concentration (Jaja *et al.,* 2002).

Protection of erythrocytes through antioxidant enzymes

Various enzymes such as superoxide dismutase, peroxidase and catalase constitute a mutually supportive team of defense against ROS. These enzymes react with reactive oxygen species and neutralize them, before they inflict damage in vital cell components. While SOD lowers the steady-state level of $O_2^{\cdot -}$, catalase (CAT) and peroxidase (POX) do the same for H_2O_2 (Bandyopadhyay *et al.,* 1999).

Results in Table 5 depicts that the changes in the activity of antioxidant enzymes decreased as a function of incubation period for both control as well as test. The POX activity at 0 h time (0.354 µmoles/mg of protein) was almost comparable in both the control and test. With the incubation period, the activity of POX decreased and the decrease was relatively much slower in the test as compared to control. The antioxidant enzyme was more active in test supplemented with 100 and 500 µg/ml of coriander than the respective control at the same time. Therefore, it can be concluded that incubation of erythrocytes with coriander extract slowed down the loss of peroxidase activity. Thus the herbal extract adds to the antioxidant defense system of erythrocytes.

Likewise, the activity of superoxide dismutase in erythrocytes was significantly higher in the test erythrocytes supplemented with coriander extract

compared to control (Table 5). The superoxide anion plays an important role in the formation of other reactive oxygen species such as hydrogen peroxide, hydroxyl radical, or singlet oxygen in living systems (Steif, 2003). SOD scavenges the superoxide radical by converting it to H_2O_2 which in turn is converted to molecular oxygen by reaction catalysed by glutathione peroxidase and CAT. The SOD activity at 0 h time (7.70 units/mg of protein) was almost comparable in both the control and test groups. As seen from results, the SOD activity displayed appreciable decrease in both the control and test group fed on coriander supplemented diet with increasing incubation period. However, decrease in SOD activity was of lower degree when erythrocytes were incubated with coriander extract as compared to its respective control.

Catalase, an enzyme ubiquitously present in all aerobic cells is a major component in primary antioxidant enzyme system. The CAT activity at 0 h time (62.6 µmoles/mg of protein) was almost same in both the control and test (Table 5). Storage of erythrocytes resulted in a significant decrease in CAT activity in both the control and test erythrocytes. However, the decrease in the catalase activity was much less in cells incubated with coriander extract, the effect being better with higher concentration (50% and 34.4% decrease in activity by 100 and 500µg/ml coriander extract respectively as compared to 63.2% decrease in control at 96h). Catalase is considered to be biologically essential in the reduction of hydrogen peroxide. Therefore it can be reasoned out that the extract of coriander at higher concentration

Table 5. Protective role of methanolic extract of coriander (local variety) on erythrocytes – Effect on antioxidant enzymes

Incubation period (h)	Control	Test Concentrations	
		100 µg/ml	500 µg/ml
Peroxidase activity (µmoles/mg of protein)			
24	0.245 ± 0.004	0.261 ± 0.011	0.343 ± 0.004
48	0.235 ± 0.007	0.249 ± 0.010	0.314 ± 0.016
72	0.218 ± 0.011	0.229 ± 0.011	0.279 ± 0.009
96	0.194 ± 0.012	0.214 ± 0.004	0.224 ± 0.011
SOD activity* (units/mg of protein)			
24	4.55 ± 0.39	6.75 ± 0.27	7.57 ± 0.43
48	3.56 ± 0.27	5.24 ± 0.26	6.08 ± 0.18
72	2.50 ± 0.35	3.84 ± 0.50	5.35 ± 0.25
96	1.32 ± 0.14	2.12 ± 0.20	4.71 ± 0.16
Catalase activity (µmoles/mg of protein)			
24	43.48 ± 1.58	50.72 ± 2.41	60.94 ± 1.84
48	40.13 ± 1.82	44.04 ± 1.64	53.51 ± 2.41
72	32.33 ± 1.37	45.15 ± 1.64	52.81 ± 1.08
96	23.06 ± 2.59	31.03 ± 3.87	41.06 ± 1.84

Values are mean ± S.D, n=3

At 0 h, activity of peroxidase = 0.354 ± 0.011 µmoles/mg of protein, activity of SOD = 7.70 ± 0.33 units/mg of protein, activity of catalase = 62.62 ± 0.95 µmoles/mg of protein.

*1 unit = Amount of enzyme that inhibits 50% of auto oxidation of pyrogallol.

(500µg/ml) offers a reasonable degree of protection against decreasing activity of antioxidant enzymes as compared to the respective controls. This data therefore suggests that coriander can help prevent oxidative stress in the alternative environment and this can be attributed to the fact that it adds to the antioxygenic potential of erythrocytes.

Conclusion

Thus, it can be concluded that methanolic extracts of coriander could inhibit the oxygen radicals better as compared to the other solvents as observed from the scavenging of superoxide, hydroxyl radical and nitric oxide radical in vitro, inhibition of lipid peroxidation in liver tissue (in vitro) as well as erythrocytes (ex vivo). Present studies also indicate that Local variety of coriander could protect the erythrocytes integrity as well as increased the resistance of the cells to loss in the activity of their antioxidant enzymes in vitro. Therefore it could be reasoned out that coriander is a good source of antioxidants and subsequently reduced the harmful effects produced by free radicals mediated oxidative stress.

References

Aebi, H. E. 1983. Catalase In :Bergmeyer H O (ed) Methods of Enzymatic Analysis **111**:273-86. Academic Press, New York.

Al-Mamary, M.A. 2002. Antioxidant activity of commonly consumed vegetables in Yemen. *Malaysian Journal of Nutrition* **2**:179-189.

Asgarpanah, J., N. Kazemivash. 2012. Phytochemistry, pharmacology and medicinal properties of Coriandrum sativum L. African *Journal of Pharmacy and Pharmacology* **6**:2340–2345.

Bandyopadhyay, U., D. Das, and R.K. Banerjee. 1999. Reactive oxygen species: Oxidative damage and pathogenesis. *Current Science* **77**(5):658-666.

Baneerjee, B.D., V. Seth, S.T. Pasher, and A.K. Chakrabarty. 1999. Biochemical effects of some pesticides on lipid peroxidation and free radical scavengers. *Toxicology Letters* **107**:33-47.

Benz, C.C., C. Yau. 2008. Ageing, oxidative stress and cancer: paradigms in parallax. *Nature Reviews Cancer* **8**:875–879.

Bishayee, S., and A.S. Balasubramaniyam. 1970. Regional lipid peroxidation in rat brain in vitro: Possible role of endogenous iron. *Journal of Neurochemistry* **18**:909-920.

Bohm, V., K. Schlesier, M. Harwat, and R. Bitsch. 2001. Comparison of different in vitro methods to evaluate the antioxidant activity with ascorbic acid, gallic acid, Trolox and uric acid as standard antioxidants. In Pfannhauser, W., G. R. Fenwick, and S. Khokhar. (Eds.), Biologically active phytochemicals in food: Analysis, metabolism, bioavailability and function (pp. 296–299). London: Royal Society of Chemistry.

Chang, S.T., J.H. Wu, S.H. Wang, P.L. Kang, N.S. Yang, and L.F. Shyur. 2001. Antioxidant activity of extracts from Acacia confusa bark and heartwood. *Journal of Agricultural and Food Chemistry* **49**:3420-3424.

Claiborne, S., and I. Fridovic. 1979. Assay for peroxidase. In: Sadasivam, S., and Manickam. (ed) Biochemical methods. pp 190. Newage International Publishers, New Delhi.

Dacie, J.V., and S.M. Lewis. 1968. Practical Haematology, 4th edition, Churchill, London.

Dariyerli, N., S. Toplan, M.C. Akyolcu, H. Hatemi, and G. Yigit. 2004. Erythrocyte Osmotic Fragility and Oxidative Stress in Experimental Hypothyroidism. *Endocrine* **25(1)**:1-6.

DeSylvia, D., M. Stuber, C.C. Fung, S. Bazargan-Hejazi, E. Cooper. 2011. The knowledge, attitudes and usage of complementary and alternative medicine of medical students. Evidence Based Complimentary and Alternative Medicine http://dx.doi.org/10.1093/ecam/nen075, 1-5.

Dragland, S., H. Senoo, K. Wake, K. Holte, and R. Blomhoff. 2003. Several culinary and medicinal herbs are important sources of dietary antioxidants. *Journal of Nutrition* **133**:1286-1290.

Dwivedi, P., and P. Kumar. 2012. Tackling the menace of obesity using botanicals having anti-obesity properties. *International Journal of Agriculture Environment and Biotechnology* **5**(2):179-185.

Esterbauer, H., K.H. Chesseman, M.U. Dianazani, G. Poli, and T.F. Slater. 1982. Separation and characterization of aldehydic products of LPO stimulated by ADP-Fe^{2+} in rat liver microsomes. *Biochemical Journal* **208**:129-140.

Etlik, O., and A. Tomur. 2006. The oxidant effects of hyperbaric oxygenation and air pollution in erythrocyte membranes (hyperbaric oxygenation in air pollution). *European Journal of General Medicine* **3**(1):21-28.

Fang, Y.Z., S. Yang, and G. Wu. 2002. Free radicals, antioxidants and nutrition. *Nutrition* **18**:872-879.

Green, L.C., D.A. Wagner, J. Glogowski, P.L. Skipper, J.S. Wishnok, and S.R. Tannenbaum. 1982. Analysis of nitrate, nitrite and (15N) nitrates in biological fluids. *Analytical Biochemistry* **126**:131-133.

Halliwell, B., J.M.C. Gutteridge, and O.I. Aruoma. 1987. The deoxyribose method: A simple "Test-tube" assay for determination of rate constants for reactions of hydroxyl radical. *Analytical Biochemistry* **165**:215-219.

Hamilton, C.A., W.H. Miller, S. AL-Benna, J. Brosnan, R.D. Drummond, M.W. McBride, and H. Dominiczak. 2004. Strategies to reduce oxidative stress in cardiovascular disease. *Clinical Science* **106**:219-234.

Jaja, S.I., A.R. Ikotun, S. Gbenebitse, and E.O. Temiye. 2002. Blood pressure, hematologic and erythrocyte fragility changes in children suffering from sickle cell anemia following ascorbic acid supplementation. *Journal of Tropical Pediatrics* **48**(6):366-370.

Kamal Eldin, A., and L.A. Appleqvist. 1996. The chemistry and antioxidant properties of tocopherols and tocotrienols. *Lipids* **31**:671-701.

Kaur, C., and H.C. Kapoor. 2002. Antioxidant, activity, total phenolic content of some Asian vegetables. *Indian Journal of Food Science and Technology* **37**:153-161.

Lavellei, V., C. Peri, and A. Rizzole. 2000. Antioxidant activity of tomato products as studied by model reactions using xanthine oxidase, myeloperoxidase and copper induced lipid peroxidation. *Journal of Agricultural and Food Chemistry* **48**:1442-1448.

Leeuwenburgh, C., and W. Heinecke. 2001 Oxidative stress and Antioxidants in exercise. *Current Medicinal Chemistry* **8**:829-838.

Lowry, O.H., N.T. Rosenbrough, A.L. Farr, and R.J. Randall. 1951. Protein measurement with folin phenol reagent. *Journal of Biological Chemistry* **193**:265-275.

Madsen, H.L., and G. Bertelsen, 1995. Spices as antioxidants. *Trends in Food Science and Technology*, **6**:271–277.

Manavalan, K., and C. Ramasamy. 2001. Physical pharmaceutics. 2nd ed. Chennai: Vignesh publishers. pp. 286.

Marcocci, L., L. Packar, D. Lefaiz, S. Gardes, and M. Albert. 1994. Antioxidant action of Ginkgo biloba extract EGb 761. *Methods in Enzymology* **234**:462-465.

Marklund, S., and G. Marklund. 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry* **47**:469-474.

Matkovics, B., M. Kotorman, I.S. Varga, D.Q. OuyHai, and C. Varga. 1998. Oxidative stress in experimental diabetes induced by streptozotocin. *Acta Physiologica Hungarica* **85**:29-38.

Mc-Cord, J.M., and I. Fridovich. 1969. Superoxide dismutase an enzymatic defense for erythrocuperin. *Journal of Biological Chemistry* **244**:6049-6050.

Melo, E.A., J.M. Filho, N.B. Guerra, and G.R. Maciel. 2003. Antioxidant activity of coriander extracts (Coriandrum sativum L). Ciencia e Tecnologia de Alimentos **23**:195-199.

Meng, Z., and W. Bai. 2004. Oxidation damage of sulfur dioxide on testicles of mice. *Environmental Research* **3**:298-304.

Mitsuda, H., K. Yasumoto, and K. Iwami. 1969. Antioxidative action of indole compounds during auto oxidation of linoleic acid. *Eryo to Shokuryo* **19**:210-214.

Momin, A.H., S.S. Acharya, A.V. Gajjar. 2012. Coriandrum sativum-review of advances in phytopharmacology. *International Journal of Pharmaceutical Sciences and Research* **3**:1233–1239.

Msaada, K., M.B. Jemia, *et al.,* 2013. Antioxidant activity of methanolic extracts from three coriander (Coriandrum sativum L.) fruit varieties. *Arabian Journal of Chemistry* http://dx.doi.org/10.1016/j.arabjc.2013.12.011 (In Press).

Naik, G.H., K.I. Priyadarsini, and H. Mohan. 2006. Free radical scavenging reactions and phytochemical analysis of triphala, an ayurvedic formulation. *Current Science* **90**(8):1100-1105.

Oganesyan, E., Z. Nersesyan, and A.Y. Parkhomenko. 2007. Chemical composition of the above-ground part of Coriandrum sativum. *Pharmaceutical Chemistry Journal* **41**:149–153.

Ohkawa, H., N. Ohishi, and K. Yagi. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric reaction. *Analalytical Biochemistry* **95**:351-358.

Ray, G., and S.A. Husain. 2002. Oxidant, antioxidants and carcinogenesis. *Indian Journal of Experimental Biology* **40**:1213-1232.

Rekha, P.S., G. Kuttan, and R. Kuttan. 2001. Antioxidant activity of Brahma rasayana. *Indian Journal of Experimental Biology* **39**:447-452.

Saffari, Y., and S.M. Sadrzadeh. 2004. Green tea metabolite EGCG protects membranes against oxidative damage in vitro. *Life Sciences* **74**(12):1513-1518.

Satyanarayana, S., K. Sushruta, G.S. Sarma, N. Srinivas, and G.V. SubbaRaju. 2004. *Journal of Herbal Pharmacology* **4**: 1-10.

Siddhuraju, P., and K. Becker. 2003. Antioxidative properties of various solvent extracts of total phenolic constituents from three different agro climatic origins of drumstick

tree leaves. *Journal of Agricultural and Food Chemistry* **51**:2144-2155.

Sreelatha, S., P. Padma, M. Umadevi. 2009. Protective effects of Coriandrum sativum extracts on carbon tetrachloride-induced hepatotoxicity in rats. *Food and Chemical Toxicology* **47**:702–708.

Steif, T.W. 2003. The physiology and pharmacology of singlet oxygen. *Med Hypotheses* **60**:567-572.

Stocks. J., and T.L. Dormandy. 1971. Autoxidation of human red cell lipids induced by hydrogen peroxide. *British Journal of Haematology* **20**:95-111.

Suboh, S.M., Y.Y. Bilto, T.A. Aburjai. 2002. Protective effects of selected medicinal plants against protein degradation, lipid peroxidation and deformability loss of oxidatively stressed human erythrocytes. *Phytotherapy Research* **18**(4): 280-284.

Subramaniam, V., M.I. Adenan, A.R. Ahmad, and R. Sahdan. 2003. Natural antioxidants: Piper sarmentosum (Kodak) and Morinda elliptica (Mengkudu). *Malaysian Journal of Nutrition* **9**(1): 41-51.

Valentao, P., E. Fernandes, F. Carvalho, P.B. Andrade, R.M. Seabra, and M.D. Bastos. 2002. Studies on the antioxidant activity of Lippia citriodora infusion: scavenging effect on superoxide radical, hydroxyl radical and hypochlorous acid. *Biological and Pharmaceutical Bulletin* **25**:1320-1323.