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Therapeutic potential of methanolic extract of coriander (*Coriandrum sativum* L.) seeds and its fractions: *In vitro* studies

Ala Vishnu Priya*, Santhosh Kumar
Vels Institute of Science,Technology & Advance Studies(VISTAS), Tamilnadu
*Corresponding Author

ABSTRACT

Methanolic extract of Coriandrum sativum (coriander) seeds was analyzed for the presence of various antioxidants; ascorbate, riboflavin, tocopherol, polyphenols and in vitro antioxidant potential. The extract, rich in polyphenolic compounds was subjected to HPLC analysis for identification and quantification of phenolics. Gallic acid, caffeic acid , ellagic acid, quercetin and kaempferol were identified. Antioxidant activity of the extract was determined by various mechanisms including DPPH free radical scavenging, metal induced protein and lipid oxidation inhibition and protection of DNA against H2O2 induced damage. Coriander had excellent free radical scavenging activity suggest that polyphenols including gallic acid, caffeic acid, ellagic acid, quercetin and kaempferol are the principle component responsible for high antioxidant activity of methanolic extract of coriander seeds. This is the first report on detailed analysis of antioxidant properties of methanolic extract of coriander seeds.

Keywords: *coriander seeds, methanol extract, polyphenols, antioxidant properties*

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INTRODUCTION

Antioxidants are believed to play a very important role in the body defense system against reactive oxygen species (ROS), which are harmful byproducts generated during normal cell aerobic respiration [1]. The radicals may cause oxidative damage by oxidizing biomolecules and results in cell death and tissue damage [2]. Atherosclerosis, cancer, emphysema, cirrhosis and arthritis have been correlated with oxidative damage [3]. Natural antioxidants in plants are related to three major groups; carotenoids, vitamins and phenolics [4]. Phenolic compounds are plant derived antioxidants that possess metal-chelating capabilities and radical scavenging properties [5].

More recent reports revealed coriander seeds to be a rich source of antioxidant compounds. Further, reports on the antioxidant properties of coriander from India are very limited. Hence, the present study was aimed to investigate the therapeutic properties of methanolic extract and fractions of methanolic extract of coriander seeds using various in vitro procedures. To investigate methanolic extract and fractions of methanolic extract of coriander seeds for therapeutic potential using in vitro methods and model systems.

MATERIAL AND METHODS

Chemicals:

All the chemicals and solvents were of analytical grade obtained from SRL, E-Merck and Sigma– Aldrich (Steinheim, Germany).

Assessment of antioxidant effects of methanolic extract and its fractions using in vitro methods

Assay of scavenging activities:

***2,2-azinobis(3-ethyl benzothiazoline-6-sulfonicacid) diamonium salt* (ABTS) scavenging assay**

ABTS radical scavenging activity was determined according to the method (6). ABTS radical cation was produced by reacting ABTS solution (7mM) with 2.45 mM ammonium persulfate and the mixture was allowed to stand in dark at room temperature 12-16 hrs before use. To 0.5 ml of test sample in different concentrations (100-500µg/ml), 0.3 ml of ABTS solution was added, volume was made up to 1 ml with ethanol, absorbance was read at 745nm and the control was maintained without sample. Butylated hydroxy toluene (BHT) was used as a positive control. Scavenging percentage was calculated by using formula given below:

% scavenging activity = (Absorbance of control) – (Absorbance of test) x 100
Absorbance of control

N, N-dimethyl-p-phenylenediamine (DMPD radical) scavenging activity

The DMPD assay was carried out by the method given by (7). DMPD (100mM) was prepared by dissolving 209 mg of DMPD in 10 ml of deionized water; 1 ml of this solution was added to 100 ml of 0.1 M acetate buffer, pH 5.25, and the colored radical cation was obtained by adding 0.2 ml of0.05 M ferric chloride (final concentration 0.1 mM). One milliliter of this solution was directly placed in cuvette and its absorbance at 505 nm was measured. Standard solutions of the Trolox were prepared as follows: 1mg/ml of Trolox was prepared by dissolving 0.1 g of Trolox in 100 ml of methanol.

A dose-response curve was derived for Trolox, by plotting the absorbance at 505 nm as percentage of the absorbance of the uninhibited radical cation solution (blank) according to the equation: inhibition of $A505$ (%) = (1- $Af/A0$)×100

2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging assay

Scavenging of DPPH radical was assayed as per the method described by Sreejayan and Rao (1996). The assay was based on the decolorization of DPPH solution and decreased absorbance at 517nm, under the influence of antioxidant substance. To 1ml of test sample at various concentrations (100- 500µg/ml) in methanol, 1ml of DPPH (0.1mM in methanol) was added, incubated at 37^{O}C for 30 min and absorbance of the test mixture was read at 517nm using cyberlab double beam spectrophotometer. Vitamin C was used as a positive control. The scavenging percentage of DPPH radical was calculated by comparing the result of the test with that of the control (1 ml methanol and 1ml DPPH) using the formula (7):

Hydroxyl radical scavenging assay

The hydroxyl radical scavenging activity was estimated by the method (8). To 1ml of test sample at various concentrations (100-500µg/ml), 1ml of iron EDTA (0.13% Ferrous ammonium sulphate and 0.26% EDTA) solution, 0.5ml EDTA solution (0.018%), 1ml of dimethyl sulfoxide (DMSO)(0.85%V/V in 0.1M phosphate buffer consisting of K2HPO4 and NaOH,pH7.4)were added and the reaction was initiated by adding 0.5ml of ascorbic acid (0.22%), incubated at 80-90^{O}C for 15 min. in a water bath and the reaction was terminated by the addition of 1ml of ice cold TCA (17.5%W/V). The reaction mixture without sample was used as control. The intensity of the colour formed was measured at 412nm against reagent blank. Trolox was used as a positive control.

Nitric oxide radical scavenging assay

Nitric oxide radical scavenging activity was estimated by the method (8). Nitric oxide (NO) radicals are generated from sodium nitroprusside solution at physiological pH. To 1ml of test sample at different concentrations (100-500µg/ml) in phosphate buffer (0.025M, pH 7.4), 1 ml of sodium nitroprusside (10mM) was added, incubated at 25^{O}C for 150 min followed by the addition of 1 ml of Griess reagent (1% sulphanilamide, 2% O- phosphoric acidand 0.1% N-(1-naphthyl) ethylene diamine] and the absorbance was read at 546nm. The reaction mixture without sample was used as control. Butylated hydroxy toluene (BHT) was used as a positive control. The nitric oxide radical scavenging percentage was calculated using the following formula:

Peroxyl radical scavenging assay

The peroxyl radical scavenging activity of various extracts of coriander seeds was estimated by the method (9). Peroxyl radical scavenging activity was measured by the inhibition pyrogallol red (PGR) oxidation by the peroxyl radicals generated from AAPH. 1 ml of 60 µM PGR was mixed with 0.1 ml of sample and 0.015 ml of 600 mM AAPH and incubated at 37^{O} C for 2 hours in a water bath. The reaction mixture was chilled immediately after incubation and the absorbance was measured at 540 nm. Reaction mixture without AAPH served as blank. The scavenging rate (SR) was calculated asfollows.

% scavenging activity = (100-[(100/(AControl blank −AControl Test)× (ASample blank −ASample Test)

Superoxide radical scavenging assay

The superoxide radical scavenging activity was determined using the method (11).To their action mixture containing 0.1ml of NBT (1mg/ml solution in DMSO) and 0.3ml of various concentrations (100-500µg/ml) of test samples in DMSO, 1ml of alkaline DMSO (1ml DMSO containing, 5mM NaOH in 0.1ml of water) was added to give a final volume of 1.4 ml and the absorbance was measured at 560nm. Control consisted of 300µl of DMSO, 0.1 ml of NBT solution and alkaline DMSO and the absorbance was measured. Butylated hydroxy toluene (BHT) was used as a positive control.

Hydrogen peroxide scavenging assay

The ability of various test samples to scavenge hydrogen peroxide was determined according to the method (12). To 500 µl of various concentrations (100-500µg/ml) of test sample, 0.6ml of 40mM hydrogen peroxide (prepared in 0.1M phosphate buffer, pH 7.4) was added, mixed, kept for 10 min, and

the absorbance was read against control containing test sample in 0.1M phosphate buffer without hydrogen peroxide at 230nm using Cyberlab double beam spectrophotometer. 6-hydroxy-2,5,7,8-tetramethyl-chromane-2-carboxylic acid (trolox) was used as a positive control.

Reducing potential

Cupric reducing antioxidant capacity *(*CUPRAC assay*)*

The cupric reducing antioxidant capacity (CUPRAC) is used for the determination of antioxidant capacity of the compounds and was carried out by the method, (13). To 400 µl of various concentrations (100-500µg/ml)of test sample, freshly prepared in DMSO($1x10^{-3}$M),1mlof10^{-2}MCuCl2, 1 ml of 7.5 x 10^{-3} M neocuproine and 1 M sodium acetate (CH_3COONH_4) solutionwere added and made up to a final volume of 4.1 ml with distilled water, incubated at room temperature for 30 min. and the absorbance at 450 nm was determined against a reagent blank/control using cyberlab double beam spectrophotometer. 6-hydroxy-2,5,7,8-tetramethyl-chromane-2-carboxylicacid(trolox)was used as a positive control.The cupric reducing antioxidant potential was calculated as follows:

$$\% \text{ reducing potential} = \frac{(\text{Absorbance of control}) - (\text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

Iron chelating capacity

The iron chelating capacity of the test samples was determined by the method(15) as given(14). To 1ml of the various concentrations of test sample(100- 500µg/ml), 0.25ml of 1mM $FeSO_4$ solution, 1ml of 0.2M Tris HCl buffer (pH 7.4), 1 ml of 2, 2' bipyridyl solution, 0.4 ml of 10% hydroxylamine HCl and 2.5ml of ethanol were added, mixed and the absorbance was measured at 522 nm. Butylated hydroxyl toluene was used as a positive control. The percentage iron chelating capacity was calculated by comparing the absorbance of the control (without the test sample) (14).

$$\% \text{ iron chelating capacity} = \frac{(\text{Absorbance of control}) - (\text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

Reducing power

Reducing power of the test samples was determined by the method (16). To 2.5 ml of test sample at different concentrations (100-500µg/ml), 2.5ml of phosphate buffer, 2.5ml of 1% potassium ferricyanide were added, incubated for 20 min at 50^0 C and 5ml of 10% TCA was added to the mixture, followed by centrifugation at 5000rpm for 10 min. The upper layer (2.5 ml) was mixed with 2.5ml of distilled water and 0.5ml of 0.1% $FeCl_3$ and the absorbance was measured at 700nm against reagent blank containing ethanol instead of the test sample. Increase in absorbance of the reaction mixture indicated the reducing power of the samples. Butylated hydroxy toluene was used as a positive control. Absorbance measured at 700nm was considered as reducing power.

RESULTS AND DISCUSSION

Free radicals are produced in human body for supply of energy, chemical signaling, detoxification process and immune function. But these free radicals also initiate the oxidation of biomolecules *viz.* proteins, lipids, nucleic acids and amino acids leading to cell injury inducing various degenerative diseases. To prevent this damage nature provides various medicinal food plants *viz.* herbs and spices rich in antioxidants which decrease the harmful effects of free radicals and therefore prevent the onset of many diseases such as cancer, cardiovascular diseases and inflammatory diseases mainly by neutralizing the reactive species by hydrogen transfer [17]. Herbs and spices contain various organic substances also called bioactive compounds like tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids. The scavenging /antioxidant activity of compounds could be due to their redox properties functioning as reducing agents, hydrogen donors. There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body and which prevent the deterioration of fats and other constituents in foodstuffs. In both cases, there is a preference for antioxidants from natural rather than from synthetic sources. There is therefore, a parallel increase in the use of methods for assessing the efficiency of such substances as antioxidants (18).

Hence, the present study was designed to assess the therapeutic potential of various solvent fractions of methanolic extract of very well known culinary spice coriander (*Coriandrum sativum* L.) which revealed interesting results as discussed under the following subheads:

Radical scavenging activities

2,2-azinobis-3-ethyl benzothiazoline-6-sulfonic acid diammonium salt (ABTS) *scavenging activity*

The ABTS radical scavenging activity of methanolic extract of coriander seeds and fractions and that of positive control BHT is presented in **Fig. 2**. The methanolic extract, all the fractions and positive control exhibited concentration dependent (100- 500µg/ml) ($p<0.001$) scavenging activity. Ethyl acetate fraction

had the least IC50 value followed by n-butanol fraction, methanolic extract, benzene, hexane and aqueous fractions. Ethyl acetate (IC50 75 µg/ml) and n-butanol (IC50 76µg/ml) fractions showed better scavenging activity than the positive control butylated hydroxy toluene (IC50 277 µg/ml). ABTS scavenging activity of methanolic extract and its fractions was found to be positively correlated ($p<0.01$) with the levels of total phenolics ($r=0.812$), flavonoids ($r=0.901$), flavonols ($r=0.761$) and tannins ($r=0.424$).The ABTS assay, which is also called the ABTS radical assay, has been widely used to evaluate antioxidant activity of components in food and beverages due to its applicability in aqueous and lipidphases (19). The original ABTS assay was based on the activation of metmyoglobin by hydrogen peroxide in the presence of ABTS (20).

Flavonoids present in various fractions of coriander seeds decolourized ABTS radical by reducing the ABTS radical cation ($ABTS^{\bullet+}$), a blue green chromogen. In the presence of antioxidants present in coriander seeds, the coloured radical was reduced back to colourless ABTS, the absorbance of which was measured at 734nm. In the present study, maximum scavenging of ABTS radicals shown by ethyl acetate fraction indicates the presence of radical scavengers viz., flavonoids, which are extracted by ethyl acetate from coriander seeds. Though slightly lesser than ethyl acetate fraction, the higher activity exhibited by n-butanol fraction is attributed to tannins that are found to be maximum in n-butanol fraction of coriander seeds (Table 1,).

N,N-dimethyl-p-phenylenediamine *(*DMPD radical*)* scavenging activity

Table 1 presents the scavenging of DMPD radical by the methanolic extract of coriander seeds and the fractions obtained in a concentration dependent manner ($p<0.001$). n-butanol fraction scavenged DMPD radicals with maximum efficiency (51- 81%, IC50 98 µg/ml) followed by ethyl acetate, benzene and hexane fractions, methanolic extract and aqueous fraction. Butylated hydroxyl toluene (BHT), a synthetic antioxidant exhibited slightly better activity (IC50 92µg/ml) than n-butanol fraction. DMPD radical scavenging activity of methanolic extract and its fractions

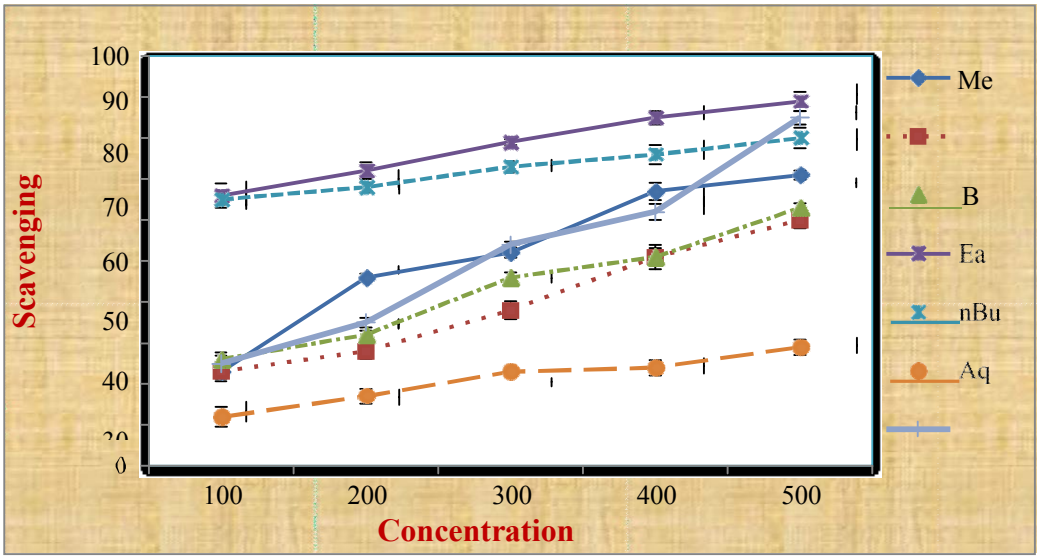

Fig. 1 ABTS radical scavenging activity (%) of methanolic extract and its fractions

Table 1: DMPD radical scavenging activity (%) of methanolic extract and its fractions

Con. (µg/ml)	Me	He	Be	Ea	nBu	Aq	BHT
100	33.0±0.2	29.5±0.7	36.4±0.4	44.5±0.4	**50.9±1.2**	26.8±0.8	**54.5±0.9**
200	36.5±0.4	38.8±1.5	42.2±1.3	54.4±0.5	**62.8±1.3**	31.6±0.8	**65.7±1.3**
300	42.7±1.9	48.2±2.3	56.7±0.2	67.6±0.2	**72.4±0.6**	38.3±0.7	**74.3±1.4**
400	57.2±0.7	57.8±0.8	61.2±0.8	71.2±0.5	**78.6±0.7**	44.6±0.4	**82.8±1.2**
500	61.8±0.9	69.6±0.4	73.6±0.5	80.7±0.3	**81.5±0.8**	59.7±0.6	**89.8±0.7**
IC50 (µg/ml)	**351**	**311**	**264**	**183**	**98**	**448**	**92**

Values are mean ± SEM of three replicates, $p<0.001$ (comparison among concentrations & among samples)

Me-methanolic extract; He-hexane fraction; Be-benzene fraction; Ea- ethyl acetate fraction; nBu-n- butanol fraction, Aq-aqueous fraction and BHT-butylated hydroxytoluene was found to be positively correlated ($p<0.01$) with the levels of total phenolics ($r=0.767$), flavonoids ($r=0.955$), flavonols ($r=0.650$) and tannins ($r=0.616$).

In the present study, scavenging of DMPD as seen by decolourisation of DMPD cation ($DMPD^{\bullet+}$) by methanolic extract of coriander seeds and its fractions indicates the presence of antioxidants which are able to transfer hydrogen atoms to $DMPD^{\bullet+}$ as stated by (18). Maximum per cent scavenging activity exhibited by ethyl acetate fraction is a result of phenolic compounds (**Table3**) present in ethyl acetate fraction. Very good activity was shown by n- butanol fraction due to tannins, a group of phenolic compounds present in n-butanol fraction, that act as antioxidants by chelating with free radicals.

2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity

2,2-diphenyl-1-picryl hydrazyl (DPPH) radical is a stable free radical. The antioxidant reacts with DPPH and converts it to 2, 2-diphenyl-1-picryl hydrazine. The DPPH radical is long-lived organic nitrogen radical with a deep purple color and the purple chromogen radical is reduced by antioxidant/reducing compounds to the corresponding pale yellow hydrazine [8]. DPPH radical scavenging activity of methanolic extract and its fractions was found to be positively correlated ($p<0.01$) with the levels of total phenolics ($r=0.855$), flavonoids ($r=0.696$), flavonols ($r=0.957$) and tannins ($r=0.196$).

Methanolic extract of coriander seeds as well as hexane, benzene, ethyl acetate, n-butanol and aqueous fractions of the methanolic extract scavenged DPPH radicals in a concentration dependant manner ($p<0.001$) (**Fig. *2*)**. Ethyl acetate fraction had shown highest DPPH radical scavenging potential ranging from 63 to 85% respectively from100 to 500µg/ml concentrations (IC_{50} 80 µg/ml). Hexane fraction showed goodscavenging potential followed by methanolic extract, benzene, n-butanol and aqueous fractions having the Lowest scavenging potential. IC_{50} values are from 80µg/ml for ethyl acetate fraction followed by 291µg/ml for hexane fraction, 357 µg/ml for methanolic extract, 383 µg/ml for benzene fraction, 415 µg/ml for n- butanol fraction and 591 µg/ml for aqueous fraction. DPPH radical scavenging efficiency was also examined for synthetic antioxidant vitamin C along with the fractions under investigation which revealed 68 to 90% scavenging efficiency with IC_{50} value of 73µg/ml meaning that ethyl acetate fraction was almost equal to the synthetic antioxidant vitamin C in scavenging DPPH radical by virtue of possessing highest content of phenolics *viz.* polyphenols, flavonoids and flavonols (Table 3).

Hydroxyl radical scavenging activity

Methanolic extract of coriander seeds and all the fractions exhibited statistically significant ($p<0.001$) hydroxyl radical scavenging activity at various concentrations i.e. 100-500 µg/ml in a dose dependant manner with IC_{50} values ranging from 183-479

µg/ml. Ethyl acetate fraction, among the different fractions, showed highest hydroxyl radical scavenging activity at all concentrations followed by hexane, benzene, methanolic extract, n-butanol and aqueous fractions. Synthetic antioxidant, 6-hydroxy- 2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) also scavenged hydroxyl radical ranging from 47 to 83% with an IC_{50} value of 105µg/ml (Table 2**)**. Hydroxyl radical scavenging activity of methanolic extract and its fractions was found to be positively correlated ($p<0.01$) with the levels of total phenolics ($r=0.761$), flavonoids ($r=0.663$), flavonols ($r=0.602$) and tannins($r=0.286$).

The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a damaging species in free radical pathology, capable of damaging almost every molecule found in the living cells [9]. Hydroxyl radicals are generally formed from the reaction of various hydroperoxides with transition metal ions such as Fenton reaction and/or iron- catalysed Haber-Weiss reaction. This radical has the capacity to react with nucleotides in DNA and causes strand breakage, which leads to carcinogenesis, mutagenesis and cytotoxicity. In addition, these species are considered to be one of the quick initiators of the lipid peroxidation process, abstracting hydrogen atoms from unsaturated fatty acids [3].

The potential of trolox in scavenging hydroxyl radical is superior to all the fractions. The highest hydroxyl radical scavenging activity at all the concentration exhibited by ethyl acetate fractions than the others is due to the higher amounts of phenolics, flavonoids and flavonols present in the ethyl acetate fraction extracted from the methanolic extract of coriander seeds as compared to the other fractions (Table 3) as phenolics are reported as hydroxyl radical scavengers [8].

Available literature reports that hydroxyl radicals are quick initiators of lipid peroxidation. Ethyl acetate fraction being a strong scavenger of hydroxyl radicals can prevent initiation of lipid peroxidation by hydroxyl radicals and is evidenced by inhibition of LPO in the *in vitro* model system *viz.* linoleic acid, hemoglobin-induced model, liver homogenate *etc.* observed in the present study in a big way by ethyl acetate fraction due to the maximum amount of polyphenolic compounds present in ethyl acetate fraction which are reported to act as potential scavengers of hydroxyl radicals [7].

As a source of scavengers of hydroxyl radicals, coriander seeds can protect biomolecules *viz.* proteins, nucleic acids and polyunsaturated fatty acids in membranes and most of the biological molecules with

which hydroxyl radicals can directly react leading to the oxidation, and thus coriander seeds prevent or decrease the rate of peroxidation chain reaction.

Nitric oxide radical scavenging activity

Methanolic extract of coriander seeds and all the fractions exhibited nitric oxide radical scavenging activity ($p<0.001$) concentration (100-500µg/ml) dependently ranging from 13% to 87% with IC_{50} values ranging from 77 to 519µg/ml, ethyl acetate (IC_{50} 77 µg/ml) fraction being the most potential and hexane (IC_{50} 519 µg/ml) fraction being the least potential out of the samples examined as depicted in **Fig. 3.** Nitric oxide radical scavenging activity of methanolic extract and its fractions was found to be positively correlated ($p<0.01$) with the levels of total phenolics ($r=0.513$), total flavonoids ($r=0.688$), total flavonols ($r=0.456$) and tannins ($r=0.551$).

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, *etc.* and is involved in the regulation of various physiological processes. Increase NO concentration may lead to nitrosylation reactions that can alter the structure of protein and inhibit their normal function. When superoxide reacts with NO it produces oxidatively active molecules i.e. peroxy nitrite ion (OONO), which causes DNA fragmentation and lipid peroxidation (10).

In the present investigation, the maximum nitric oxide radical scavenging activity exhibited by ethyl acetate fraction, followed by n-butanol, benzene, aqueous fractions, methanolic extract and hexane fractions can be attributed to various active principles reported (www.ars_grin.gov) as well as identified to be present in coriander seeds, which can be supported by the higher potential to scavenge nitric oxide radicals than the synthetic antioxidant butylated hydroxy toluene (IC_{50} 176 µg/ml). Nitric oxide is reported to play a dual role i.e., stimulating and inhibiting lipid peroxidation (11). In the present study, inhibitory effect of fractions on lipid peroxidation in different model systems examined, revealed anti lipid peroxidative effect of coriander seeds. By scavenging nitric oxide radicals, the bioactive compounds present in coriander seeds, would have contributed to the inhibition of lipid peroxidation in various model systems. In a similar way, coriander seeds can inhibit lipid peroxidation in the living systems by scavenging nitric oxide radicals. Our observation can be supported by previous *in vivo* experimental evidences that *Coriandrum sativum* decreased nitric oxide level in arthritis patients (11), indicating that the seeds of *Coriandrum sativum* contains flavonoids (Table 1**,**) that scavenge nitric oxide and thus could be beneficial in the treatment of radical-induced diseases.

Peroxyl radical scavenging activity

Methanolic extract and all the fractions scavenged peroxyl radicals in accordance with the concentrations ($p<0.001$) with scavenging potential ranging from 12-85% and IC_{50} values ranging from 145-423µg/ml. Ethyl acetate fraction had the highest scavenging potential (IC_{50} 145µg/ml) and benzene had the lowest scavenging potential with highest IC_{50} value (423µg/ml). All the test samples examined, had lesser potential than that of positive control, butylated hydroxy toluene which exhibited highest scavenging activity with IC_{50} value of 87 µg/ml (Table 3). Peroxyl radical scavenging activity of methanolic extract and its fractions was found to be positively correlated ($p<0.01$) with the levels of total phenolics ($r=0.801$), total flavonoids ($r=0.639$), total flavonols ($r=0.380$) and tannins ($r=0.294$).

In the present study, ethyl acetate fraction scavenged peroxyl radicals generated by 2,2′- azobis (amidinopropane) dihydrochloride (AAPH) in the reaction system better than all the other fractions due to the presence of phenolic compounds such as flavonoids and tannins (Table 1 and 3) present in coriander seeds as phenolic compounds are reported to possess antioxidant activity which is mainly due to their redox properties that can play an important role in neutralizing free radicals, quenching singlet and triplet oxygen and decomposing peroxides [20]. This is also supported by inhibition of lipid peroxidation in the *in vitro* model systems (Table 6) by ethyl acetate fraction of coriander seeds confirming the presence of anti lipid peroxidative compounds viz. phenolic compounds in coriander seeds.

Superoxide radical scavenging activity

Table 4 depicts that all the fractions dose dependently and significantly ($p<0.05$) scavenged superoxide radical starting from 100-500 µg/ml with IC_{50} value ranging from 257-620µg/ml, the lowest being for ethyl acetate fraction. As a whole, superoxide radical scavenging activity was below 65% for all the test samples. However, among all the fractions, ethyl acetate fraction exhibited better activity than all the others followed by methanolic extract, n-butanol, aqueous, benzene and hexane fractions. Per cent superoxide radical scavenging activity of the synthetic antioxidant, positive control, BHT, ranged from 37-84% with IC_{50} value of 210µg/ml, which is better than that of all the fractions and methanolic extract of coriander seeds. Super oxide radical scavenging activity of methanolic extract and its fractions was found to be positively correlated ($p<0.01$) with the levels of total phenolics ($r=0.676$), total flavonoids ($r=0.505$), total flavonols ($r=0.489$) and tannins($r=0.002$).

Although superoxide anion is a weak oxidant, it is the most common free radical *in vivo,* which is a precursor for the other reactive oxygen species such as singlet oxygen that have the potential reactivity with biological molecules and thus, induce tissue damage and lead to oxidative stress. Superoxide has also been observed to directly initiate lipid peroxidation. NBT assay is carried out to test whether the extracts scavenge superoxide anions or not. Alkaline DMSO, used as a superoxide generating system, reacts with NBT to give coloured formazan [11].

Comparatively higher superoxide radical scavenging activity shown by ethyl acetate fraction of coriander seeds, though to a lesser extent, as compared to the positive control, BHT indicates the presence of phenolic compounds such as flavonoids, flavonols and tannins extracted into ethyl acetate (which could have scavenged superoxide radicals, because phenolic compounds are reported to quench oxygen.

In the present study, fractions of methanolic extract of coriander seed are identified to possess superoxide radical scavenging activity at all concentrations, though with a degree difference, can prevent lipid peroxidation and is evidenced by inhibition of lipid peroxidation in *in vitro* model systems in the same study.

However, comparatively higher superoxide radical scavenging activity exhibited by ethyl acetate fraction is supported by inhibition of lipid peroxidation in linoleic acid model system, hemoglobin- induced system, in liver homogenate by ethyl acetate fraction. Coriander seeds having superoxide radical scavengers, can act as inhibitors of peroxidation and protect from stress-induced diseases as superoxide radicals have been reported to directly initiate lipid peroxidation, a marker of oxidative stress, a root cause of number of diseases [12].

In oxidative stress conditions caused due to the free-radicals *viz.* superoxide radicals, hydroxyl radicals *etc.* sequential formation of noxious molecules occurs leading to hemolytic effects. Oxidative damage often results in a clinical manifestation of mild to severe hemolysis in patients with these disorders. In some cases, the release of hemoglobin during hemolysis and subsequent therapeutic transfusion lead to systemic iron overload that further potentiates the generation of ROS [12].

In the present study, maximum superoxide radical scavenging activity exhibited by the ethyl acetate fraction as compared to other fractions coincides with higher antihemolytic activity (Fig. 13) shown by ethyl acetate fraction in the same study. This indicates ethyl acetate extractives as powerful superoxide radical scavengers as well as inhibitors of hemolysis as evidenced in the present study.

Hydrogen peroxide scavenging activity

Fig. 4 shows concentration dependent hydrogen peroxide scavenging activity of methanolic extract and fractions of methanolic extract of coriander seeds. Ethyl acetate fractions scavenged hydrogen peroxide very efficiently (IC_{50} 99µg/ml), better than all the other fractions as well as the synthetic antioxidant, trolox, followed by benzene, hexane, n-butanol fractions, methanolic extract and aqueous fractions. Hydrogen peroxide scavenging activity of methanolic extract and its fractions was positively correlated ($p<0.01$) with the levels of total phenolics ($r= 0.873$), total flavonoids ($r= 0.875$), total flavonols ($r= 0.721$) and tannins ($r= 0.006$).

Hydrogen peroxide (H_2O_2) is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide is naturally produced in organisms as a byproduct of oxygen metabolism. Almost all living organisms possess enzymes known as peroxidases, which harmlessly and catalytically decompose low concentrations of H_2O_2 to water and oxygen (12). Hydrogen peroxide can easily cross the cell membrane and attack different sites converting itself into water. It can cause DNA damage in the form of both single and double strand breaks which is believed to be the initial step in the induction of cancer [13]. Membrane lipids are rich in unsaturated fatty acids and are most susceptible to oxidative processes. Especially, linoleic acid and arachidonic acid are targets of lipid peroxidation [14].

Maximum potential of scavenging hydrogen peroxide (Fig. 4) by ethyl acetate fraction is the result of phenolic, flavonoids, triterpenoids present in ethyl acetate fraction of methanolic extract of coriander seeds. Hydrogen peroxide is not very reactive but it can cause cytotoxicity by giving rise to hydroxy radicals leading to oxidation of unsaturated fatty acids in the membranes resulting in the peroxidation of lipids [13]. In the present study, all the fractions and methanolic extract could scavenge hydrogen peroxide in the reaction system indicating that all fractions have hydrogen peroxide scavenging compounds as a result of which all the fractions of coriander seeds could effectively inhibit peroxidation in liver homogenate (Fig. 8) taken as a model system. So, coriander seeds by virtue of a number of phytochemicals, can control lipid peroxidation of the cell membrane and protect the cells from oxidative damage.

Reducing potential

Cupric reducing antioxidant capacity (CUPRAC)

The assay, cupric reducing antioxidant capacity (CUPRAC) is based on the reduction of Cu (II) to Cu (I) by reluctant (antioxidants) present in the sample as shown by methanolic extract and fractions of methanolic extract of coriander seeds is displayed in Table 5. All the fractions showed significantly ($p<0.05$) high cupric reducing ability ranging from 18% to 88% at the concentrations of 100-500 µg/ml. The sequence of cupric reducing capacity of fractions and methanolic extract of coriander seeds is as follows:

Ethyl acetate (IC_{50} 78 µg/ml) > n-butanol (IC_{50} 207µg/ml) > benzene (IC_{50} 268µg/ml) and aqueous (IC_{50} 311µg/ml) > methanolic (IC_{50} 313 µg/ml) > hexane (IC_{50} 504µg/ml). Trolox examined as positive control in the same study, exhibited CUPRAC starting from 60 to 84% with IC_{50} value of 83µg/ml which is more than that of ethyl acetate fraction indicating lesser potential than ethyl acetate. Cupric reducing antioxidant capacity of methanolic extract and its fractions was found to be positively correlated ($p<0.01$) with the levels of total phenolics ($r=0.625$), total flavonoids ($r=0.586$), total flavonols ($r=0.612$) and tannins($r=0.409$).

Cupric reducing antioxidant capacity (CUPRAC) assay uses the copper (II)- neocuproine reagent as the chromogenic oxidant. It has been introduced as Bioxytech AOP-490 and CUPRAC developed by Apak *et al*., (2008). This is a novel antioxidant measurement of the CUPRAC chromophore, Cu(I)- neocuproine (Nc) chelate, formed as a result of the redox reaction of antioxidants with the CUPRAC reagent, Cu (II) neocuproine, where absorbance was recorded at the maximal absorbance wavelength of 450nm [4].

The data generated in the present investigation with respect to cupric reducing capacity of methanolic extract of coriander seeds revealed interesting results. Ethyl acetate fraction exhibited highest cupric reducing capacity with lowest IC_{50} value (78µg/ml), which correlates with the highest quantity of polyphenolic compounds present in ethyl acetate fraction.

Iron chelating capacity

Fig. 5 shows iron chelating activity of methanolic extract and fractions of methanolic extract of coriander seeds. All the fractions exhibited concentration dependency ($p<0.001$) in chelating iron. Ethyl acetate fraction had the highest capacity with an IC_{50} value of 82 µg/ml followed by n-butanol fraction with IC_{50} value of 303µg/ml, methanolic extract with IC_{50} value of 368 µg/ml aqueous fraction with IC_{50} value of 458µg/ml whereas, benzene and hexane fractions had very less iron chelating potential with IC_{50} values of 491 and 552 µg/ml. The ethyl acetate fraction had shown higher iron chelating capacity than the synthetic antioxidant quercetin (IC_{50} 97µg/ml) used as a positive control and all the other fractions. Iron chelating activity of methanolic extract and its fractions was found to be positively correlated ($p<0.01$) with the levels of total phenolics ($r=0.767$), total flavonoids ($r=0.626$), total flavonols ($r=0.524$) and tannins($r=0.002$).Iron is essential for life because it is required for oxygen transport, respiration, and activity of many enzymes. In complex systems, such as food and food preparations, various mechanisms may contribute to oxidative processes, such as Fenton reaction, where transition metal ions play a vital role. Different reactive oxygen species might be generated and various target structures such as lipids, proteins, and carbohydrates, can be affected. If they undergo the Fenton reaction, these reduced metals may form highly reactive hydroxyl radicals, and thereby ,lead to oxidative stress [16].

The resulting oxy radicals cause damage to cellular biomolecules and lead to cellular impairment. Since ferrous ions are the most effective pro-oxidants in food systems, the good chelating effect would be beneficial, and removal of free iron ion from circulation could be a promising approach to prevent oxidative stress-induced diseases. When iron ion is chelated, it may lose pro-oxidant properties. Iron, in nature, can be found as either ferrous (Fe^{2+}) or ferric ion (Fe^{3+}), with the latter form predominant in foods. Ferrous chelation may render important antioxidative effects by retarding metal-catalyzed oxidation [17].

The transition metal, iron, is capable of generating free radicals from peroxides by Fenton reactions and may be implicated in human cardiovascular diseases. Because $Fe2^{+}$also has been shown to cause the production of oxy-radicals and lipid peroxidation, minimizing $Fe_{2^{+}}$ concentration in Fenton reactions affords protection against oxidative damage. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of other chelating agents, the complex formation is disrupted with the result that the red colour of the complex decreases [18].

In the present study, the highest activity exhibited by ethyl acetate fraction can be attributed to the phytochemicals such as phenolics, flavonoids, terpenes, phytosterols and tannins (Table 2 and3,) present in coriander seeds which help iron lose its pro-oxidant properties by chelating the ferrous ion thus retard its oxidation and also inhibits the formation of peroxides by Fenton reaction.

Reducing power

Figure 7 depicts reducing power of methanolic extract and fractions of methanolic extract of coriander

seeds. Among the fractions, ethyl acetate fraction showed the highest reducing power, followed by n-butanol and aqueous fractions, methanolic extract, benzene and hexane fractions wherein IC_{50} values ranged from77-289 µg/ml. Thus, all the fractions, dose dependently exhibited reducing power ($p<0.001$). Ethyl acetate fractions exhibited better reducing power than the synthetic antioxidant BHT (IC_{50} 102 µg/ml). Reducing power of methanolic extract and its fractions was found to be positively correlated ($p<0.01$) with the levels of total phenolics ($r=0.597$), total flavonoids ($r=0.552$), total flavonols ($r=0.415$) and tannins ($r=0.247$).The ability to reduce of ferric ion (Fe^{3+}) in the reaction mixture to ferrous (Fe^{2+}) is a significant indicator of antioxidant activity which is generally associated with the presence of reductones [19]. The yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of each extract. The presence of antioxidants in the extract causes the reduction of Fe^{3+}/ferriccyanide complex to ferrous form. Therefore, Fe^{2+}complex can be monitored by measuring the formation of Perl's Prussian blue at 700nm [20].

The reducing ability of a compound may serve as a significant indicator of its antioxidant potential. All the fractions as well as methanolic extract of coriander seeds had shown 34 to 89% of reducing power at various concentrations indicating the presence of various antioxidants extractable in different solvents, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued abstraction and reductive capacity and radical scavenging. The results show that the extract contains hydrophilic polyphenolic compounds (Table 1-3,), that exhibit greater reducing power. Inhibition of lipid peroxidation in hemoglobin-induced system, in liver homogenate by various extracts with a difference is because of the variation in the extraction of reducing compounds in to various extracts present in coriander seeds.

CONCLUSION

The present study reveals that coriander seed is a rich source of natural antioxidants which could be extracted efficiently with methanol. Polyphenolic compounds of the methanol extract of coriander seeds include gallic acid, caffeic acid, ellagic acid, quercetin and kaempferol. The extract exhibited good free radical scavenging property and could protect lecithin, protein and DNA against metal ion induced oxidation and per oxidation. Potential use of coriander as an antioxidant neutraceutical and as food preservative needs to be explored further.

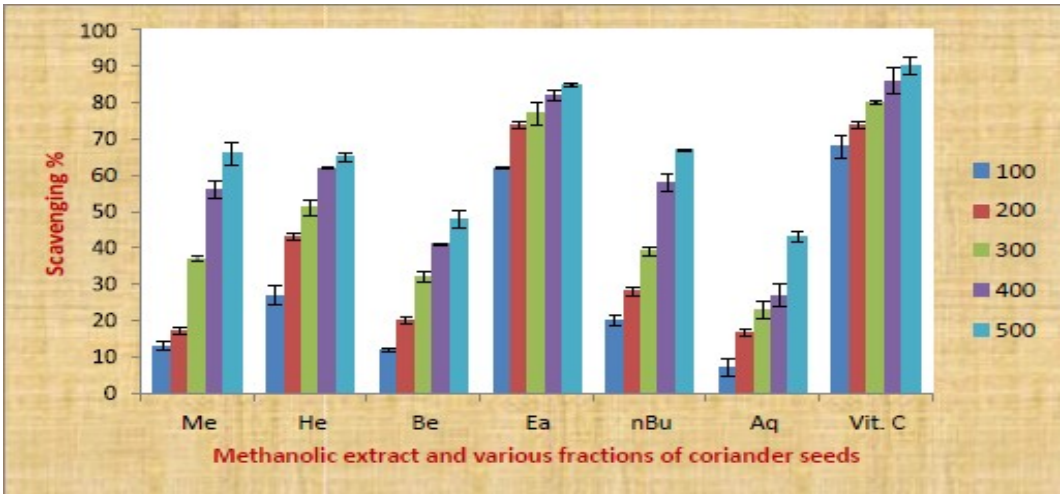

Fig. *2* DPPH radical scavenging (%) activity of methanolic extract and its fraction

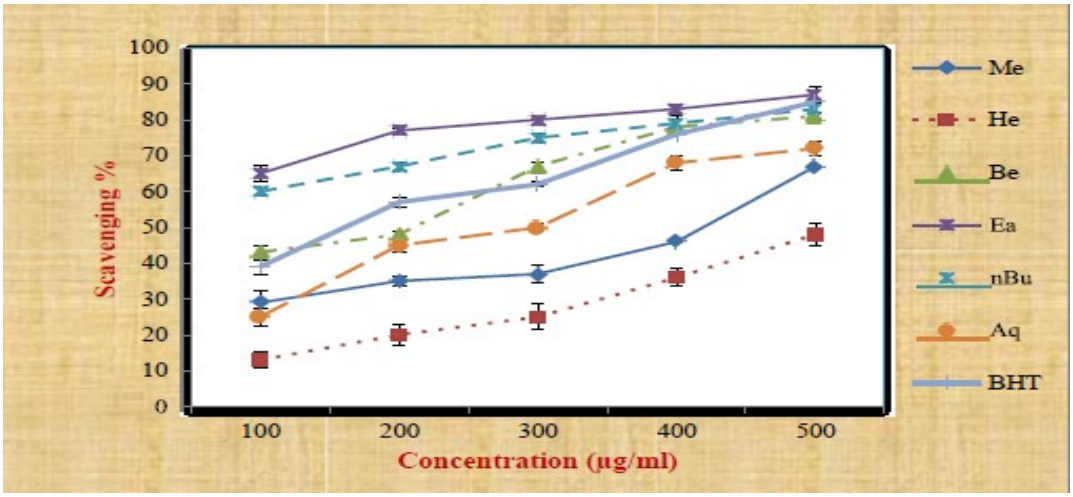

Fig. 3 Nitric oxide radical scavenging (%) activity of methanolic extract and its fractions

Table 2: Hydroxyl radical scavenging (%) activity of methanolic extract and its fractions

Con. (µg/ml)	Me	He	Be	Ea	nBu	Aq	Trolox
100	25.7±0.4	33.9±0.3	30.1±1.5	**40.2±1.9**	25.2±0.3	20.2±1.4	**47.4±0.4**
200	35.9±1.7	52.5±0.7	47.5±0.3	**54.1±0.8**	37.2±0.5	25.4±0.6	**58.4±0.9**
300	47.9±0.7	61.7±0.3	55.8±0.7	**67.5±0.5**	45.5±0.2	30.4±0.7	**66.3±0.4**
400	55.8±0.9	65.5±0.5	69.4±0.1	**72.1±0.8**	59.4±1.6	48.2±1.2	**78.3±0.9**
500	67.9±1.8	70.2±0.4	76.1±1.1	**80.8±0.3**	68.5±1.7	52.2±1.4	**82.7±0.5**
IC50	**313**	**192**	**210**	**183**	**337**	**479**	**105**

Values are mean ± SEM of three replicates, p<0.001 (comparison among concentrations & among samples)
Me-methanolic extract; He-hexane fraction; Be-benzene fraction; Ea- ethyl acetate fraction; nBu- n- butanol fraction and Aq-aqueous fraction

Table 3 :Peroxyl radical scavenging (%) activity of methanolic extract and its fractions

Con. (µg/ml)	Me	He	Be	Ea	nBu	Aq	BHT
100	12.2±0.3	24.5±0.7	15.4±1.0	**46.2±1.6**	24.3±1.4	26.6±1.6	**55.6±2.3**
200	14.3±1.6	45.3±0.9	24.8±1.4	**55.5±2.4**	39.7±1.5	39.3±1.5	**65.4±1.5**
300	34.6±1.7	55.3±1.4	35.6±1.4	**68.3±0.8**	49.4±1.3	48.9±0.8	**79.3±2.3**
400	55.4±0.9	65.7±1.7	46.3±0.7	**76.8±0.9**	58.8±0.6	59.4±1.6	**81.6±1.3**
500	69.1±1.4	80.3±2.3	58.2±2.2	**85.3±1.7**	67.7±0.8	62.4±1.2	**88.2±1.8**
IC50 (µg/ml)	**334**	**256**	**423**	**145**	**304**	**324**	**87**

Values are mean ± SEM of three replicates, p<0.001
(comparison among concentrations & among samples)
Me-methanolic extract; He-hexane fraction; Be-benzene fraction; Ea- ethyl acetate fraction; nBu-n-butanol fraction; Aq-aqueous fraction and BHT-butylated hydroxy toluene

Table 4: Superoxide radical scavenging (%) activity of methanolic extract and its fractions

Con.	Me	He	Be	Ea	nBu	Aq	BHT
100	7.8±0.4	2.8±0.2	5.1±0.1	**39.2±0.7**	6.5±0.5	7.2±0.1	**37.1±1.5**
200	16.8±0.8	17.9±0.5	20.1±0.4	**43.4±1.2**	22.3±1.2	16.8±0.5	**47.5±2.0**
300	17.1±0.6	20.5±0.4	22.6±0.9	**58.4±0.3**	24.3±0.2	18.3±0.8	**69.1±0.9**
400	24.1±0.5	25.2±0.9	24.5±0.4	**60.2±1.7**	28.7±0.6	26.6±2.4	**79.2±0.8**
500	46.4±0.7	40.3±0.6	41.2±0.2	**62.3±0.5**	44.3±0.2	42.3±1.6	**83.5±1.6**
IC50	**539**	**620**	**607**	**257**	**564**	**591**	**210**

Values are mean ± SEM of three replicates, p<0.001
(comparison among concentrations & among samples)

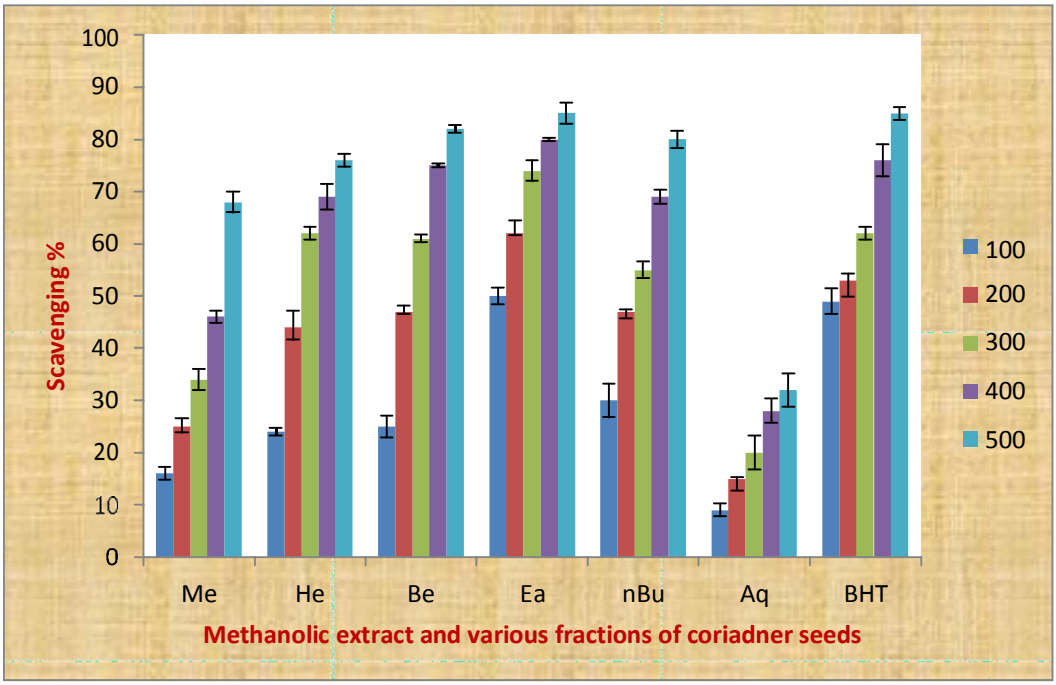

Fig. 5 Hydrogen peroxide scavenging (%) activity of methanolic extract and its fractions

Table 5: Cupric reducing antioxidant capacity (CUPRAC)(%) of methanolic extract and its fractions

Concentration	Me	He	Be	Ea	nBu	Aq	Trolox
100	35.5±0.8	17.6±0.3	30.4±1.5	**63.9±1.2**	40.2±0.9	23.8±0.3	**60.2±0.3**
200	44.6±1.3	21.4±0.5	47.8±0.5	**70.7±0.4**	48.2±0.6	34.8±1.5	**67.2±0.5**
300	47.7±0.7	26.6±0.7	55.9±1.5	**80.7±0.5**	55.5±0.4	48.2±0.9	**75.5±1.2**
400	55.4±0.5	38.9±0.4	61.8±0.8	**84.8±0.4**	79.4±1.0	74.3±1.8	**79.4±1.6**
500	62.6±1.2	49.6±0.2	75.4±0.9	**87.5±1.5**	83.5±0.8	87.1±0.8	**83.5±1.7**
IC50	**313**	**504**	**268**	**78**	**207**	**311**	**83**

Values are mean ± SEM of three replicates, p<0.05
(comparison among concentrations & among samples)

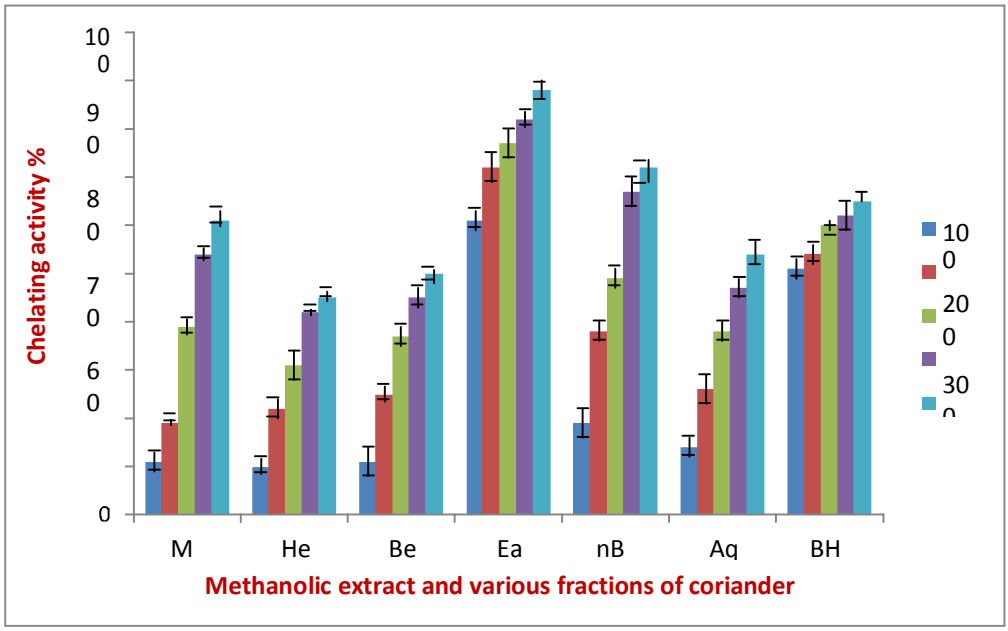

Fig. 5 Iron chelating activity (%) of methanolic extract and its fractions

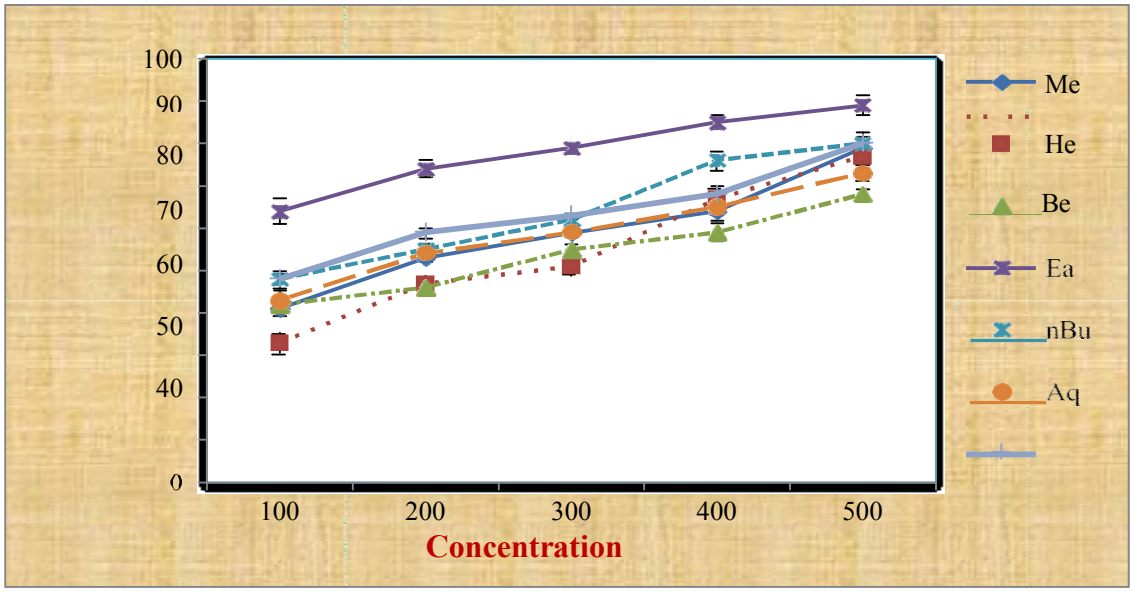

Fig. 6 Reducing power of methanolic extract and its fractions

Me-methanolic extract; He-hexane fraction; Be-benzene fraction; Ea- ethyl acetate fraction; nBu-n-butanol fraction; Aq-aqueous fraction and BHT-butylated hydroxy toluene

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