INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

ANTIOXIDANT PROFILE OF *CORIANDRUM SATIVUM* METHANOLIC EXTRACT

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Article Received on: 10/02/14 Revised on: 21/02/14 Approved for publication: 07/03/14

DOI: 10.7897/2230-8407.050347

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 (18.696 ± 0.12 mg/g dry seeds) was subjected to HPLC analysis for identification and quantification of phenolics. Gallic acid (173.656 µg), caffeic acid (80.185 µg), ellagic acid (162.861 µg), quercetin (608.903 µg) and kaempferol (233.70 µg)/g dry seeds 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 H_2O_2 induced damage. Coriander had excellent free radical scavenging activity with IC_{50} value 0.4 mg dry seed weight, whereas comparatively higher IC_{50} was observed with metal ion chelating assays (7.2-8.0 mg dry seed weight). The results 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 composition and antioxidant properties of methanolic extract of coriander seeds.
Keywords: coriander seeds, methanol extract, polyphenols, antioxidant properties

INTRODUCTION

The survival on oxygen has presented a serious challenge to the aerobes, since ROS are the normal by-products of cell respiration and metabolism. Biological systems are especially sensitive to reactive oxygen species (ROS), the reactive forms of oxygen which arise either as by-products of oxidative phosphorylation in the mitochondria, or as the result of exposure to environmental chemicals and toxins. The biological macromolecules including proteins, lipids and nucleic acids, are vulnerable to oxidative attack. ROS can disturb homeostasis of cells and tissues, which ultimately threatens the integrity of the organism. Acute stress responses are characterized by the cessation of cell division, degradation of irreparably damaged proteins or organelles by proteasomal and autophagic mechanisms. Oxidative stress is reported to play an important role in ageing and various clinical disorders such as diabetes, atherosclerosis, reperfusion injury, cancer[1] etc. Humans and other mammals possess a multitude of cytoprotective mechanisms against environmental and biochemical damage. One general mechanism that cells employ to protect themselves against the oxidative damage is to maintain a reducing intracellular milieu, by keeping a significant concentration of reducing equivalents in the form of reduced glutathione, thioredoxin, and other redox buffers[2,3]. Dietary intake of naturally occurring antioxidants such as ascorbic acid, Vitamin E and phenolic compounds have ability to reduce oxidative damage associated with many diseases including cancer, cardiovascular diseases, cataract, arthritis, diabetes[4]. Due to several side effects of synthetic antioxidants, such as risk of liver damage and carcinogenesis in laboratory animal, there is a need for more effective, less toxic and cost effective natural antioxidants. Medicinal plants appear to have these desired comparative advantages and are rich source of bioactive principles that form the ingredients in traditional systems of medicine, modern medicines, pharmaceutical intermediates, neutraceuticals and food supplements. Coriander (*Coriandrum sativum*) is an annual herb of the family Apiaceae. The herb is cultivated and used extensively in Russia, Europe, India, Turkey, Argentina and United States of America. The leaves and dry fruits of coriander are used as spice in various food preparations. Traditionally coriander seeds (dry fruits) are also used to cure indigestion, cough, bronchitis, vomiting, diarrhea and dysentery, against worms, rheumatism and joint-pain[4]. The coriander seeds are reported to affect carbohydrate[5] and lipid metabolism[6]. Antioxidant activity of aqueous extract of coriander on carotene and linoleic acid oxidation has been studied[7]. Wong and Kitts (2006) have reported free radical scavenging and antibacterial activity in the extracts of coriander leaves and stem[8]. Free radical scavenging and lipid per oxidation inhibition activity in the dichloromethane and aqueous extracts of coriander leaves and seeds has also been reported by Wangensteen, *et al*.[9] The present study estimates the level of various bioreactive antioxidant compounds in the methanol extract of coriander seeds. The study further evaluates the free radical scavenging and possible protection of macromolecules proteins, lipids and DNA against oxidative stress by the extract.

MATERIALS AND METHODS

The coriander (*Coriandrum sativum*) seeds, procured from the local market, were identified and authenticated at Department of Botany, Kurukshetra University, Kurukshetra, India. Diphenyl-picrylhydrazyl (DPPH), acetonitrile, gallic acid, Folin-Ciocalteau reagent and methanol were purchased from Hi-media, Mumbai, India. Bovine serum albumin, calf thymus DNA, 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB), thiobarbituric acid, caffeic acid, ellagic acid, ferulic acid, quercetin and kaempferol were purchased from Sigma Chemical Company, USA. All other chemicals used were of analytical grade.

Extraction

Coriander seeds were dried at 60°C and ground to get fine powder. Ground coriander seeds were shaken with 80 % methanol (1 g/10 ml) in a shaker at room temperature for 4 h followed by re-extraction of the residue for 2 h. Collected extract was filtered through double layered muslin cloth and centrifuged to get clear supernatant. Extract was concentrated in a vacuum evaporator and stored at -20°C and used after appropriate dilutions for various experiments.

Antioxidant analysis

Coriander seed extract was analyzed for ascorbate by diluting in 5 % metaphosphoric acid in presence of 10 % stannous chloride and adding equal volume of 2 % thiourea in 5 % HPO_3. After incubating at 37°C for 6 h the contents were chilled and 5 ml of 85 % H_2SO_4 was added slowly. The absorbance was read at 540 nm against reagent blank after 30 minutes[10]. A calibration curve of ascorbic acid (1-20 µg/ml) was prepared. To determine total ascorbic acid, reduced ascorbate was first oxidized by adding bromine water. Riboflavin content of coriander extract was estimated after diluting with 0.2M acetate buffer pH 4.0. Few drops of caprylic alcohol and 3 ml of 4 % potassium permanganate solution (freshly prepared) were added. Within 2 minutes, 3 ml of H_2O_2–water solution (1:1) was added and pH adjusted to 7.0 with NaOH. The fluorescence by the filtrate was measured at 530 nm with excitation at 470 nm using fluorescence spectrophotometer[10]. Standard riboflavin (1 µg/ml) was used for calibration purpose. For tocopherol estimation, the extract was mixed with saturated potassium hydroxide and hexane. Hexane layer was evaporated under nitrogen and dissolved in ethanol. To the ethanol extract, 0.2 ml of 2 % bathophenanthroline followed by 0.2 ml of ferric chloride reagent was added in dark. After 1 minute, 0.2 ml of 0.01M phosphoric acid (prepared in alcohol) was mixed and read at 534 nm. Standard DL-tocopherol (1-10 µg) was used to prepare the calibration curve. Total polyphenolic content of the methanolic extract of coriander was estimated by Folin-Ciocalteau method[11]. Aliquot of the extract was mixed with 2 ml of sodium carbonate (2 %). After 2 minutes, 100 µl of Folin-Ciocalteau reagent (IN) was added and absorbance was read at 750 nm after 30 minutes. The methanolic extract was defatted with n-hexane. The defatted extract was treated with 2N HCl to hydrolyze glycosidic bonds. The extract was dried, again dissolved in methanol and subjected to HPLC for qualitative and quantitative analysis of free phenolic compounds by modifying the method given by Ani *et al.*[11] The HPLC system (Agilent Technologies Company) was equipped with dual lamp binary system, UV detector, C18 column (i.d. 4.6 mm × 150 mm, 5 µm) and data was integrated by Agilent Chem Station software. Standards and sample extracts were analyzed using the following gradient program (A, 100 % acetonitrile B, HPLC Grade Water: 0 minute, 5 % A: 10 minutes, 15 % A: 20 minutes, 25 % A: 30 minutes, 35 % A: 40 minutes, 45 % A: 50 minutes, 55 % A). Flow rate was 0.5 ml/min and injection volume was 10 µl. Peak area (280 nm) of the sample was used as an index of the amount of component and the retention time of individual peaks was used to identify polyphenols by comparing with standard polyphenols – gallic acid, caffeic acid, ellagic acid, ferulic acid, quercetin and kaempferol.

Measurement of free radical scavenging activity

To 1 ml of DPPH solution (50 x 10^{-5} M) different dilutions of the coriander extract were added in a final volume of 1.1 ml. The decrease in absorbance due to the scavenging of DPPH radicals by the extract was recorded at 517 mm[11] after 5 minutes. The percentage inhibition of DPPH scavenging with different dilutions of extract was calculated and IC_{50}, the concentration at which 50 % of the initial DPPH could be scavenged was noted.

$$\% \text{ inhibition} = [(\Delta Abs_{control} - \Delta Abs_{sample})/\Delta Abs_{control}] \times 100$$

Estimation of lipid and protein oxidation Inhibition

The amount of malonaldehyde produced by copper induced egg lecithin per oxidation was monitored as thiobarbituric acid reacting substances (TBARS) to measure lipid per oxidation as described earlier[10]. The coriander extract were added to the reaction mixture containing lecithin and $CuCl_2$ in 50 mM Tris-HCl buffer (pH 7.4) and incubated at 37°C for 15 minutes. Malonaldehyde produced was determined by adding TBA reagent containing 0.37 % thiobarbituric acid (TBA), 15 % trichloroacetic acid (TCA), 0.04 % butylated hydroxyl toluene (BHT) and 2 % ethanol. Mixture was heated at 100°C for 15 minutes and centrifuged at 3000 g for 10 minutes. The absorbance of supernatant at 535 nm was recorded. IC_{50}, the concentration inhibiting 50 % of per oxidation was calculated. Oxidative modifications in BSA were induced by copper in presence and absence of different dilutions of coriander extract[10]. The reaction mixture containing albumin (10 mg/ml) and $CuCl_2$ in 50 mM Tris–HCl buffer (pH 7.4) was incubated at 37°C for 2 h. Phosphate buffer (pH 8.0) containing 12.5 mM EDTA plus 10.0 M urea and phosphate buffer (pH 7.0) containing 10 mM 5,5-dithio-bis (2-nitrobenzoic acid) was added to the reaction mixture. The absorbance was recorded at 412 nm as an index of cysteine-SH residues. Percent inhibitory ratio was calculated as follows:

$$\% \text{ inhibition} = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$$

Inhibition of oxidative damage to DNA

Oxidative damage is induced in DNA by hydroxyl radicals generated by Fenton's reaction[11]. The reaction mixture containing 3 µg of calf thymus DNA in 20.0 mM phosphate buffer saline (pH 7.4) and different concentrations of the extract (0.5, 1.0, 1.5 and 2.0 µg) in a final volume of 9 µl was pre-incubated for 15 minutes. The oxidation was induced by adding 1.0 mM $FeSO_4$ + 10.0 mM ascorbic acid and incubated for 1 h at 37°C. The loading buffer (xylenecyanol, 0.25 %; bromophenol blue, 0.25 % and glycerol 30 %) was added and the mixture was subjected to gel electrophoresis in 1.5 % agarose-TAE buffer system and run at 60 V. DNA was visualized and photographed by using UV- transilluminator (Genei) and Chemidoc (Biorad) system to assess the damage and protection.

RESULTS AND DISCUSSION

Antioxidants are the chemical moieties which inhibit the production and propagation reactions of ROS or terminate these reactions when present in small amounts. Ascorbate, riboflavin, tocopherol and polyphenols have redox potential high enough to scavenge or terminate ROS[2-4]. Polyphenols can contribute as metal ion chelators due to the presence of various hydroxyl radicals. The π electron cloud of one or more benzene rings makes them suitable as antioxidants[13].

Table 1: Antioxidant Profile of Coriander Seed Extract

Compound	Amount (µg/g dry wt.)
Oxidized ascorbate	150.5 ± 9.14
Reduced ascorbate	136.6 ± 9.36
Total ascorbate	287.1 ± 1.82
Riboflavin	4.67 ± 0.37
Tocopherol	181.33 ± 9.02
Total Polyphenols	18.696 ± 0.12*
Gallic acid	173.656
Caffeic acid	80.185
Ellagic acid	162.861
Quercetin	608.903
Kaempferol	233.700

*(mg/g dry wt.)

Table 2: Antioxidant Activity of Coriander Seed Extract Determined by Different Assays (As Percent Inhibition of Control)

Assay	Dry Weight (mg)									
	0.25	0.5	1.0	2	3	4	5	6	8	10
DPPH Scavenging	30.56	52.36	65.55	78.35	80.43	83.47	85.60	86.10	ND	ND
Lipid per oxidation	0	0	0	1.32	10.36	21.45	31.21	39.84	49.71	61.25
Protein oxidation	0	0	2.32	5.21	7.30	10.36	15.63	22.03	64.15	84.13

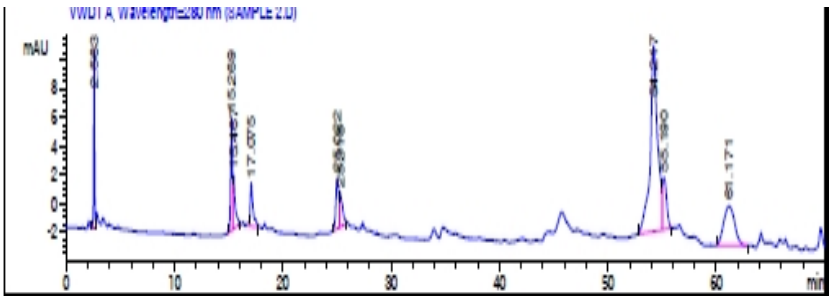

Figure 1: HPLC analysis of the coriander extract; peak at retention time 2.583, 15.269, 25.022, 54.217 and 61.171 minutes are identified as gallic acid, caffeic acid , ellagic acid, quercetin and kaempferol respectively

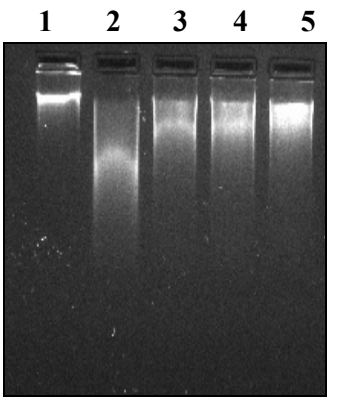

Figure 2: Protection of DNA from H_2O_2 induced damage in presence of extract equivalent to different amounts of coriander seeds; Lane1-control DNA (1.5 µg); Lane2-DNA + Fenton's reagent with ascorbic acid; Lane3-coriander (0.5 µg) + DNA + Fenton's reagent with ascorbic acid; Lane 4-coriander (1.0 µg) + DNA + Fenton's reagent with ascorbic acid; Lane5-oriander (1.5 µg) + DNA + Fenton's reagent with ascorbic acid

To identify and determine the level of possible antioxidants in coriander seeds, the seed extract was analyzed for the presence of biomolecules, known to have antioxidant activity. The coriander seeds had low methanol extractable ascorbate, riboflavin and tocopherol (Table 1). Total ascorbate and tocopherol are 287.1 µg and 181.33 µg/g dry seeds, where as riboflavin is only 4.67 µg/g dry seeds. However, the extract had considerable amount of polyphenols 18.696±0.12mg GAE/g dry weight of seeds. Polyphenols have good antioxidant potential both as free radical scavenger and inhibitor of metal induced oxidation[13]. Wangensteen *et al.* have reported that butanol and ethyl acetate extracts of coriander seeds had polyphenols 1.16 g GAE and 0.189 g GAE/100 g dry seeds[9]. Higher amount of polyphenols from coriander seeds were extracted here using methanol. Polyphenolic compounds are usually present as glycosides in plant sources. The coriander seed extract was therefore, hydrolyzed with 2N HCl to break glycosidic bonds before analysis by HPLC (Figure 1). The identification of polyphenols was done by comparing retention time of the peaks with that of standard compounds. Coriander seed extract contained gallic acid, caffeic acid, ellagic acid, quercetin and kaempferol (Table 1). Quantification of the identified compounds was achieved by comparing the peak area of individual compound with that of standards (2 ng/10 µl). Methanolic extract of coriander had 173.656 µg gallic

acid, 80.185 µg caffeic acid, 162.861 µg ellagic acid, 608.903 µg quercetin and 233.70 µg kaempferol/g coriander seeds. Extract of fresh coriander leaves and stem is reported to contain caffeic acid, protocatchunic acid, chlorogenic acid, ferulic acid and flavanols such as quercetin[7]. Shan *et al*. have identified quercetin, isoquercetin, rutin and their glucuronoid derivatives from coriander seeds but quantitative analysis of polyphenols from coriander seeds has not been reported[14]. Antioxidant activity of the polyphenols increases with the number of hydroxyl groups and the density of π electron cloud. Gallic acid, among the simple phenolics and quercetin among the flavanols are the most potent antioxidants[13]. Presence of high amount of quercetin and kaempferol, along with other polyphenols indicates high efficacy of the spice as an antioxidant. Scavenging free radicals such as hydroxyl or superoxide radicals and terminating chain reaction, chelating metal ions and inhibiting ROS production, donating electrons or hydrogen to terminate chain reactions are some of the ways by which antioxidants reduce oxidation. The mode of action of natural antioxidants may be varied and could involve multiple mechanism of action. Tocols and phenols act as primary antioxidants while ascorbic acid may reductatively regenerate oxidized primary antioxidants. The antioxidant activity of a natural source is generally related to either of these activities or as a synergist. Synergism between various antioxidants has been reported[15].

Antioxidant activity of coriander seed extract was examined by methods based on different principles i.e. DPPH free radical scavenging, copper induced lecithin peroxidation, copper induced cysteine oxidation in BSA and peroxide induced damage to DNA. DPPH is a stable free radical which can absorb an electron or hydrogen to become a stable diamagnetic molecule. Scavenging of these free radicals by the antioxidants in coriander seed extract was observed as decrease in optical density of the reaction mixture. Coriander extract exhibited a concentration dependent elimination of DPPH free radicals (Table 2). Methanolic extract corresponding to 5 mg of coriander seeds caused complete scavenging of free radicals. IC_{50} of the spice is 0.4 mg for DPPH free radical scavenging activity. These results indicate that antioxidants in coriander seeds are effective electron or hydrogen donors and this activity contributes to the antioxidant capacity of coriander seeds. Ramadan *et al*. have found a positive correlation between the radical scavenging activity and polyphenol content of the chloroform extracts of various spices[16]. Free radical scavenging activity of aqueous[7], ethanol and ethylacetate[9] extracts of coriander seeds is also positively correlated to the polyphenol content of the extracts. Polyphenols from other spices are also reported to have DPPH free radical scavenging activity[8,10,11,17].

Metal ions such as iron and copper can induce oxidation of lipids leading to the production of per oxy radicals, which in turn propagate chain reaction and accelerate lipid oxidation. Lipid oxidation brings about chemical changes, spoiling the fats and fatty acids of foods. Cellular membranes being rich in polyunsaturated fatty acids are easily attacked by free radicals. Oxidative damage to the membrane lipids affects their permeability and induces apoptosis, autogenesis, carcinogenesis[18] and the processes related to membrane integrity. Malonaldehyde produced by copper induced oxidation of lecithin in presence and absence of different dilutions of coriander extract was determined as thiobarbituric acid reactive substances (Table 2). In controls, 19.35 ± 0.318 nmoles of MDA was produced and the production of MDA was reduced to 11.64 ± 0.776 nmoles in presence of the extract equivalent to 6 mg of the coriander seeds indicating 40 percent inhibition of lipid per oxidation. IC_{50} calculated from the data is 8.0 mg. Higher concentrations of spice extract are required for metal chelation as compared to free radical scavenging. The results indicate that antioxidants from this spice are efficiently preventing the oxidation of lipids induced by metals either by metal chelation or by inhibiting the propagation reactions being hydrogen/electron donor. A decrease in lipid oxidation in presence of coriander[7,8], parsley[8], cumin[11] and fenugreek[17] extracts has been reported. This activity has been attributed to the metal chelating property of the polyphenols of the extracts of spices and herbs[14].

Oxidation of the sulphydryl groups of cysteine to cystine may cause changes in the structure and functions of the proteins. These proteins may be enzymes, hormones or components of immune system. Deleterious impact of oxidative stress in biological systems is related to the damage of proteins, enzymes and various transcriptional factors like Nrf2, NFkB and AP-1[19,20]. BSA was subjected to oxidative modifications by incubation with copper ions in presence and absence of coriander extract (Table 2). Although little effect was observed with lower concentrations of the extract, the process of oxidative modifications was inhibited by 84 % in presence of extract equivalent to 10 mg of coriander seeds. Presence of extract equivalent to 7.2 mg spice is enough to inhibit the metal induced protein oxidation up to 50 %, which is comparable to IC_{50} for copper induced lecithin oxidation, indicating that this is the influence of metal chelating components of the extract. Methanol extracts of various herbs and spices are reported to exhibit metal chelating activity comparable to EDTA. Coriander extract inhibited metal induced oxidation up to 88 % at 400 ppm concentration[21]. Metal ion chelating capacity plays a significant role in antioxidant mechanism since it reduces the concentration of the oxidation catalyzing transition metal in lipid and protein oxidation. Oxidation of DNA and RNA by hydroxyl radicals can cause mutations[22]. Guanosine is oxidized to hydroxyl-2-deoxyguanosine and thymine is modified to thymine glycol under oxidative stress caused by carcinogens[23]. Oxidative damage to DNA is shown to be extensive and could be a major cause of physiological changes associated with aging and degenerative diseases such as cancer, cardiovascular diseases, immune-system decline, diabetes mellitus etc. Antioxidants are believed to decrease the attacks on DNA by free radicals and thus, protect against mutations that cause disease status[24]. Oxidative stress generated by Fenton`s reaction can cause breaks in calf thymus DNA and can uncoil the super coiled DNA. Incubation of DNA with $FeSO_4$ and ascorbate has caused damage to DNA and damaged DNA moves to a greater extent in the gel (Figure 2). Presence of extract equivalent to 1.0 µg and 1.5 µg coriander in the incubation mixture could prevent the damage. This protective impact of the extract indicates that antioxidant formulation from coriander seeds can efficiently quench hydroxyl radicals from the reaction mixture and protect nucleic acid from oxidative damage.

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.

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Cite this article as:
Dua Anita, Agrawal Sharad, Kaur Amanjot, Mahajan Ritu. Antioxidant profile of Coriandrum sativum methanolic extract. Int. Res. J. Pharm. 2014; 5(3):220-224 http://dx.doi.org/10.7897/2230-8407.050347

Source of support: Nil, Conflict of interest: None Declared