

ANNALS OF PHYTOMEDICINE An International Journal

ISSN 2278-9839

Phytochemicals and medicare potential of ethyl acetate fraction of methanolic extract of coriander (Coriandrum sativum L.) seeds

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Received August 27, 2013: Revised September 30, 2013: Accepted October 10, 2013: Published online December 30, 2013

Abstract

Phytochemicals provide protection against stress-induced diseases as they adopt multimodal therapeutic approach against multifactorial pathogenecity of diseases, *viz.* diabetes, (controlling blood glucose and lipids), cancer (inhibition of one or more of the stages of cancer process) and inflammatory diseases [inhibition of proinflammatory enzymes such as lipoxygenase (LPO), cyclo-oxygenases (COX-1 and COX-2)]. Coriander is one of the oldest spices, possessing multiple traditional health benefits. The present investigation was aimed to identify the phytochemicals by qualitative and quantitative tests and also to assess medicare potential of coriander. Methanolic extract of coriander seeds and all the fractions possessed phytochemicals in an individualized manner but ethyl acetate fraction had the highest concentration of phenolic compounds, steroids and terpenoids, glycosides etc. Hence, ethyl acetate fraction was evaluated for radical scavenging, antiperoxidative and anti-inflammatory activities, using in vitro methods and model systems. Ethyl acetate fraction of coriander seeds concentration dependently scavenged ABTS (IC50 75µg/ml) and DPPH (IC50 80µg/ml) radicals, exhibited antiperoxidative effect in linoleic acid model system (IC₅₀105µg/ml) and liver homogenate (IC_{so}100µg/ml) and showed significant anti-inflammatory activity by inhibiting the activities of inflammatory enzymes, viz. lipoxidase (IC_{s0} 97µg/ml) and xanthine oxidase (IC_{so}206 µg/ml). High performance thin layer chromatography (HPTLC) of ethyl acetate fraction revealed the presence of many phenolic compounds, out of which quercetin and rutin could be identified and quantified; rutin being predominant in the fraction, followed by quercetin. Thus, coriander (Coriandrum sativum L.) seeds are a promising source of phytochemicals with wide applications in the prevention and treatment of diseases induced by free radicals.

Key words: *Coriandrum sativum*, High performance thin layer chromatography (HPTLC), flavonoids, quercetin, rutin.

Introduction

Plants are storehouses of natural products which differ widely in their structures, biological properties and mechanism of action. For long, medicinal plant research was limited to isolation of new compounds from plant species, carried out mostly by chemists; pharmacologists used to test their therapeutic efficacy. However, in the current fast-changing global scenario, the medicinal plant research has shifted largely to botanists with reference to authentication of botanical identity of drugs, standardization of techniques for cultivation of medicinal plants, and analysis of the impact of environmental degradation/climate change on their active ingredients. There has been increasing interest in the research on phenolic compounds from dietary sources, due to growing evidences of their versatile health benefits. Many herbs and

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spices have been a major source of treatment for human diseases since time immemorial. The World's one fourth population is dependent on traditional medicines, particularly plant drugs for curing various ailments, proving that there is much more to get from herbs and spices than mere culinary function as seasonings, used to improve sensory properties of food (Iqbal, 2013).

Many attempts of modern scientists are directed towards finding evidences of various bioactivities, identification of responsible compounds and their molecular targets (Tapsell et al., 2006). The medicinal value of plants lies in some chemical substances that produce a definite physiological action in the human body. The most important of these bioactive constituents of plants are alkaloids, phenolic compounds, viz. tannins, flavonoids. Many of the indigenous medicinal plants are used as spice plants and food plants (Okwu, 2001). Coriander is one of the oldest spices, used as traditional medicine with multiple health benefits. In addition to the polyphenolic compounds, methanolic extract of coriander seeds contains compounds which prevented lysis of RBCs (Rajeshwari et al., 2012). Hence, phytochemicals in coriander seeds are of great importance in the development of pharmaceuticals, cosmetic and food products and also to improve the health status (Rajeshwari and Andallu, 2013).

In order to study the therapeutic effects of various phytochemical compounds present in herbs and spices, it is necessary to extract them from the source prior to the analysis. Extraction of compounds in plant materials is influenced by their chemical nature, the extraction method employed, particle size, storage time and conditions, as well as presence of interfering substances. Phenolic extracts of plant materials are always a mixture of different classes of phenolics that are soluble in the solvent system used. Additional steps may be required for the removal of unwanted phenolics and nonphenolic substances such as waxes, fats, terpenes and chlorophylls (Naczk and Shahidi, 2004). Hence, the present study was aimed to sequentially fractionate methanolic extract of coriander seeds using solvents of increasing polarity, viz. hexane, benzene, ethyl acetate, n-butanol and water; to examine the fractions for the presence of bioactive compounds using standard qualitative tests for carbohydrate-derivatives, amino acids, organic acids, tannins, glycosides, saponins, triterpenoids, flavonoids and flavones and quantitative tests for various phenolic compounds and to evaluate the best fraction for radical scavenging, antiperoxidative and anti-inflammatory activities using in vitro methods and model systems.

Material and Methods

Preparation of coriander seed extract and evaluation of medicare potential

Coriander seeds (*Coriandrum sativum* L.) were purchased in one lot from local departmental stores, dried, powdered and

extracted with 80% methanol(Me), thrice (1:1, w/v), at room temperature (Petra *et al.*, 1999). The combined extract was concentrated in a vacuum evaporator and the residue was dissolved in water and fractionated successively with hexane (He), benzene (Be), ethyl acetate (Ea), n-butanol (nBu) and water (Aq) and each extract was evaporated to dryness. Before use, a small amount of each fraction was re-dissolved in various solvents as required, at a concentration of 1mg/ml (Hashim *et al.*, 2005).

Qualitative analysis of phytochemicals

Preliminary phytochemicals screening for carbohydratederivatives, amino acids, organic acids, tannins, glycosides, saponins, triterpenoids, flavonoids and flavones in the methanolic extract of coriander seeds and various fractions was carried out using standard qualitative methods (Edeoga *et al.*, 2005).

Quantitative analysis of phytochemicals

All the fractions were analyzed for total polyphenolics (Singleton and Rossi, 1965), total flavonoids (Chang *et al.*, 2002), total flavonols (Kumaran and Karunakaran, 2006) and tannins (Price *et al.*, (1978).

Assessment of medicare potential using in vitro methods and models

The ABTS (Re *et al.*, 1999) and DPPH (Sreejayan and Rao, 1996) radical scavenging activities, antiperoxidative activities in linoleic acid model system (Kikuzaki and Nakatani, 1993) and in liver homogenate (Yue *et al.*, 1995) and the anti-inflammatory activities using soybean lipoxidase (Shinde *et al.*, 1999) and xanthine oxidase (Bondet *et al.*, 1997) inhibitory effects were evaluated for ethyl acetate fraction, selected based on qualitative and quantitative analyses.

Identification of the phytochemicals using high performance thin layer chromatographic technique (HPTLC).

The ethyl acetate fraction rich in the flavonoids was initially subjected to qualitative TLC analysis followed by observation under UV light (366 nm), spraying with ferric chloride reagent and further subjected to qualitative HPTLC for confirmation. The HPTLC screening was carried out using "CAMAG[®] Linomat V" sample applicator, "CAMAG[®]TLC3" densitometric scanner and "CAMAG[®] WinCATs" software (CAMAG, Switzerland, Version 1.2.3) on pre-coated HPTLC plates.

The ethyl acetate fraction was concentrated by evaporating the solvent, and made up to 10ml in standard flask, applied on (E. Merck) aluminium plate pre-coated with silica gel 60 F254 of 0.2 mm thickness. 5% methanol in chloroform was standardized to be used as the solvent system. An amount of 5 μ L to 30 μ L of sample was manually applied on TLC plates in a band-shape of 1cm. The TLC plates were dried and run 8 cm. After air drying the plate was visualized in UV at 254 and 366 nm for the spots.

Statistical Analysis

The results obtained were subjected to two-way analysis of variance (ANOVA) and the significance of the difference between means was calculated. Values expressed are mean of three independent samples analyzed in triplicate \pm standard error of means (SE_x) (SPSS version 15.0).

Results and Discussion

The results of phytochemical screening are presented in Table 1. All the fractions responded positively for qualitative tests for various phytochemicals, *viz*. carbohydratederivatives, tannins, triterpenoids, flavonoids, while only a few fractions responded positively for glycosides. However, ethyl acetate fraction possessed highest amount of carbohydrate-derivatives, phenolic compounds, flavonoids, glycosides and alkaloids; n-butanol fraction had the highest amount of tannins reported to possess strong anti-radical activities (Rajeshwari *et al.*, 2012)

Phenolic compounds in methanolic extract and other fractions of coriander seeds

Polyphenolic compounds, extensively disseminated in plants, known to be excellent antioxidants, possess the capacity to scavenge free radicals generated due to oxidative processes and protect antioxidant defenses in the body. Phenolic **compounds**, *viz.* total phenolics, flavonoids, flavonols, tannins estimated in methanolic extract, hexane, benzene, ethyl acetate, n-butanol and aqueous fractions of methanolic extract of coriander seeds are presented in Table 2.

Ethyl acetate fraction had the highest phenolic content followed by n-butanol, hexane, methanolic extract, benzene and aqueous fractions. Flavonoids were also concentrated in ethyl acetate fraction followed by n-butanol, hexane, benzene, methanolic extract and aqueous fractions. Flavonol content was more in ethyl acetate followed by n-butanol, methanolic extract, hexane, benzene and aqueous fractions. n-butanol fraction had maximum tannin content followed by

Phytochemicals	Methanolic extract	Hexane fraction	Benzene fraction	Ethyl acetate fraction	n-butanol fraction	Aqueous fraction
Carbohydrate-derivatives	++	+	-	+++	++	+
Phenolic compounds	++	++	+	+++	++	++
Tannins	+	+	-	+	+++	+
Flavonoids	+	+	-	++	+	+
Steroids	-	-	+	-	-	-
Terpenoids	-	-	++	-	-	-
Saponins	+	+	+	+	+	+
Glycosides	++	-	-	++	++	-
Alkaloids	+	+	+	++	++	+

Table 1: Phytochemicals in methanolic extract and various fractions of methanolic extract of coriander seeds

Table 2: Phenolic compounds in methanolic extract and various fractions of methanolic extract of coriander seeds

Sample extract	Total phenolic (mg/100g) [Gallic acid equivalents (GAE)]	Total flavonoids (mg/100g) [Rutin equivalents (RE)]	Total flavonols (mg/100g) [Rutin equivalents (RE)]	Tannins (mg/100g) [Catechin equivalents (CE)]
Methanol	2.77±0.40	0.51±0.09	1.40±0.02	0.76±0.04
Hexane	5.63±0.20	2.21±0.05	1.35±0.04	0.02±0.04
Benzene	2.66±0.06	1.63±0.03	0.64±0.02	0.45±0.10
Ethyl acetate	7.77±0.13	3.40±0.10	1.80±0.06	0.39±0.06
n-butanol	5.76±1.07	3.30±0.09	1.60±0.07	0.80±0.08
Aqueous	2.54±0.02	0.50±0.04	0.26±0.03	0.24±0.03

Values are Mean \pm S.E.M. of three replicates

methanolic extract, benzene, ethyl acetate, aqueous and hexane fractions. The variation in the phytochemicals in methanolic extract and other fractions is due to the variation in their solubility in the solvents of varying polarity (Table 2).

ABTS [2, 2-azinobis(3-ethyl benzothiazoline-6-sulfonicacid) diamonium salt] radical scavenging activity

Figure 1 shows ABTS radical scavenging potential of ethyl acetate fraction of methanolic extract of coriander seeds and that of positive control butylated hydroxy toluene. Ethyl acetate fraction exhibited significantly (p<0.001) higher ABTS radical scavenging potential with IC_{50} value of 75µg/ml than the positive control butylated hydroxy toluene (IC_{50} 277 µg/ml).



Figure 1: ABTS radical scavenging activity of ethyl acetate fraction of methanolic extract of coriander seeds. Values are Mean \pm SE_x of three replicates, **p<0.001

The ABTS radicals are scavenged by antioxidants via the mechanism of electron/hydrogen donation and are assessed by measuring the decrease in the absorbance at 414 nm (Chen and Yen, 2007). In the present study, ethyl acetate fraction of coriander seeds scavenged ABTS radicals with 66 to 89% potential at various concentrations (100-500µg/ml). The decolourisation assay using free blue green ABTS⁺ radicals was very useful tool in expeditiously measuring the antioxidant activity of ethyl acetate fraction of coriander seed extract representative of various compounds. Significant scavenging potential exhibited by ethyl acetate fraction shows efficacy of polyphenolic compounds that are extracted in to ethyl acetate confirming polyphenols, viz. flavonols, flavonoids, tannins (Table 2) to be efficient scavengers of free radicals such as ABTS than the synthetic antioxidant butylated hydroxy toluene.

DPPH [2, 2- diphenyl-1-picryl hydrazyl] radical scavenging activity

The ethyl acetate fraction of coriander seeds scavenged (p<0.001) DPPH radicals in a concentration dependant manner ranging from 62 to 85% respectively at 100 to 500µg/ml with

 IC_{50} value of 80 µg/ml (Figure 2). DPPH radical scavenging efficiency of antioxidant vitamin C (positive control) ranged from 68% to 90% with IC_{50} value of 73 µg/ml indicating that ethyl acetate fraction is similar to vitamin C in scavenging DPPH radicals by virtue of highest phenolics, *viz.* polyphenols, flavonoids, flavonols and tannins present in the extracts of coriander seeds (Tables 1 and 2).



Figure 2: DPPH radical scavenging activity of ethyl acetate fraction of methanolic extract of coriander seeds. Values are Mean \pm SE_x of three replicates, **p<0.001

DPPH is a useful radical for investigating the scavenging activities of phenolic compounds and a substrate to evaluate the antioxidative activity of antioxidants (Duh *et al.*, 1999). The reduction in the absorption is indicative of the capacity of the ethyl acetate fraction to scavenge radicals independent of any enzymatic activity. The essence of DPPH method is that the antioxidants react with the stable free radical, *i.e.* 2, 2-diphenyl-1-picryl hydrazyl (deep violet colour) and convert it to 2,2-diphenyl-1-picryl hydrazine with discolouration. The degree of discolourisation indicates the scavenging potential of the antioxidants present in the sample (Hossain and Rahman, 2011).

In the present study, the DPPH radical scavenging potential shown by ethyl acetate fraction can be attributed to the highest polyphenols, flavonoids and flavonols present in ethyl acetate fraction of coriander seeds (Tables 1 and 2) indicating that coriander seeds are rich source of phenolic compounds that can scavenge free radicals.

Inhibition of lipid peroxidation in linoleic acid emulsion system

Ethyl acetate fraction of methanolic extract of coriander seeds significantly (p<0.001) inhibited peroxidation of linoleic acid in the model system at all the concentrations (100-500 μ g/ml), in a concentration dependant manner with IC₅₀ value of 105 μ g/ml (Figure 3).



Figure 3: Lipid peroxidation inhibitory effect in linoleic acid emulsion system by ethyl acetate fraction of methanolic extract of coriander seeds

Values are Mean \pm SE_x of three replicates, **p<0.001

The inhibition of peroxidation of linoleic acid in the emulsion system also called as ferric thiocyanate (FTC) method, measures the amount of peroxide in the beginning of the reaction, where ferric ion was formed upon reaction of peroxide with ferrous chloride. The ferric ion will then unite with ammonium thiocyanate producing ferric thiocyanate, a red colored substance. The darker the color, the higher will be the absorbance (Manian *et al.*, 2008).

In the present study, inhibition of oxidation of linoleic acid in the reaction system is a reflection of the complexity of the extract composition (aqueous/hydrophobic nature of compounds) as well as potential interaction between the extract and emulsion component, oil, water or lipid; air interfaces as reported by Koleva *et al.* (2002) for antioxidant activity of *Camellia sinensis* (L.) O. Kuntz, *Ficus bengalensis* L. and *Ficus racemosa* L. Significant inhibition of lipid peroxidation (LPO) in the reaction system exhibited by ethyl acetate fraction in the present study is a reflection of highest phenolic compounds (flavonoids and flavonols) in ethyl acetate fraction (Table 2) which are more efficient than the synthetic antioxidant, quercetin used as a positive control in this investigation.

Inhibition of lipid peroxidation in liver homogenate

The ethyl acetate fraction of coriander seeds significantly (p<0.001) inhibited FeSO₄-induced lipid peroxidation (LPO) in the liver homogenate (IC₅₀ value of 100 µg/ml) (Figure 4).

Lipid peroxidation is initiated by reactive oxygen species (ROS). Some typical ROS are superoxide (O₂•), singlet oxygen ¹O₂, triplet oxygen (³O₂), ozone (O³), hydroxyl radical (•OH), alkoxyl radical (RO•), and peroxyl radical (RO•). Once these free radicals are formed, lipid peroxidation progresses and, consequently, lipids produce various so-called secondary oxidation products, some of which have been used as biomarkers to investigate their role in the diseases such as cancer, diabetes, arthritis *etc*. In biological systems, oxidative

degradation of poly unsaturated fatty acids (PUFA) in cell membranes generates a number of degeneration products, such as malondialdehyde (MDA), which is found to be an important cause of the cell membrane destruction and cell damage (Yoshikawa *et al.*, 1997).



Figure 4: Lipid peroxidation inhibitory effect of ethyl acetate fraction of methanolic extract of coriander seeds in liver homogenate

Values are Mean ± S.E.M. of three replicates, **p<0.001

Iron-induced lipid peroxidation in liver homogenate, used as a model system to examine anti-lipid peroxidative effect of ethyl acetate fraction of coriander seed extracts in the present investigation, is a well validated system for generating ROS as reported by Gutteridge (1995). Although a number of other *in vitro* assays are useful to assess antioxidant potential of plant extracts in terms of inhibition of LPO, only this assay involves biological tissue which has a considerable amount of historical control data.

In the present study, ethyl acetate fraction of methanolic extract of coriander seeds inhibited (72%) FeSO4-induced lipid peroxidation (a marker of oxidative stress in liver homogenate). Iron, a transition metal is capable of generating free radicals from peroxides by the Fenton reaction and is implicated in many human diseases (Halliwell and Gutteridge, 1990). Fe²⁺ has also been shown to produce oxyradicals and lipid peroxides, so the decrease of Fe²⁺ concentration in the Fenton reaction would protect against oxidative damage. The results of the present study, revealed the presence of potent antioxidants such as polyphenols, flavonoids, flavonols etc. in ethyl acetate fraction that could efficiently inhibit FeSO,induced lipid peroxidation in liver homogenate, since polyphenols are also known for their ability to prevent peroxidation of fatty acids and provide a defense against oxidative stress caused by oxidizing agents and free radicals (Slusarczyk et al., 2009).

It is interesting to note that ethyl acetate fraction of methanolic extract of coriander seeds exhibited better antiperoxidative efficacy than the positive control, butylated hydroxy toluene indicating the presence of more efficient antioxidants in coriander extract and /or synergistic action of various antioxidants present in coriander seeds.

Anti-inflammatory effects

Soybean lipoxidase (LOX) inhibitory effect

In the present study, the anti-inflammatory activity in terms of inhibition of soybean lipoxidase (LOX), exhibited by ethyl acetate fraction of coriander seeds is depicted in Figure 5. Highly significant (p<0.001) inhibitory activity (51 to 82%) was shown by ethyl acetate fraction with IC_{50} value of 97 µg/ml and is almost similar to that of Quercetin, a positive control, which showed inhibition of 50 to 79% at various concentrations with an IC_{50} value of 99 µg/ml.



Figure 5: Soybean lipoxidase inhibitory activity of ethyl acetate fraction of methanolic extract of coriander seeds Values are Mean \pm SE_x of three replicates, **p<0.001

The inhibition of lipoxidase/lipoxygenase at the progression of inflammation and cancer led the researchers to the development of drugs targeting their activity. LOX inhibitors possess antiproliferative effects against various cancer cells and thereby have protective effect against various cancer types (Leone *et al.*, 2007). It is worth commenting that ethyl acetate fraction containing the highest concentration of phenolics, *viz.* flavonoids, flavonols (Tables 1 and 2) exhibited almost similar LOX inhibitory effect as that of standard, quercetin, which is an interesting finding in the study, reflecting the efficient fractionation of the phytochemicals present in coriander seeds. Polyphenolic compounds present in the ethyl acetate fraction, the most effective radical scavengers and reducing agents act as lipoxygenase inhibitors as reported by Xanthopoulou *et al.* (2009).

Xanthine oxidase inhibitory effect

Ethyl acetate fraction of methanolic extract of coriander seeds showed significant (p<0.001) inhibitory activity (30% to 77%)

with perfect proportionality to the concentration (Figure 6) with IC_{50} value of 206 µg/ml. Allopurinol, effectively inhibits xanthine oxidase and decreases production of uric acid. In the present study, allopurinol used as a positive control exhibited xanthine oxidase inhibition, ranging from 47% to 82% with IC_{50} of 106µg/ml, which was higher than that of ethyl acetate fraction.



Figure 6: Xanthine oxidase inhibitory effect of ethyl acetate fraction of methanolic extract of coriander seeds Values are Mean \pm SE_x of three replicates, **p<0.001

Xanthine oxidase (XO), a flavoprotein formed from xanthine dehydrogenase, under oxidative conditions catalyses the oxidation of hypoxanthine to xanthine and generates superoxide and uric acid. The accumulation of uric acid leads to hyperuricemia and gout and hence, inhibitors of uric acid formation could be useful as therapeutic agents for these diseases. In addition, a large amount of superoxide anion generation by xanthine oxidase leads to peroxidative damages of cells, and inhibitors of the generation and/or scavengers of superoxide anion are useful in the prevention of oxidative damages (McCord, 1985). Ethyl acetate fraction exhibited good x0 inhibitory activity by virtue of high amount of polyphenolic compounds present in ethyl acetate fraction as flavonoids are reported to be strong inhibitors of xo, a molybdenum containing enzyme. However, inhibitory activity exhibited by coriander was lesser than that of positive control allopurinol.

The inhibitory action of flavonoids may be by way of competitively binding xanthine binding site on xanthine oxidase thereby inhibiting the activity of xanthine oxidase as reported by Lin *et al.* (1999). The flavonoids quercetin, myricetin, rutin, kaempferol, luteolin are reported to efficiently inhibit xanthine oxidase by binding with xanthine binding site of the enzyme (Masuoka and Kubo, 2004). As coriander seeds are reported (www.ars-grin.gov/duke.) to contain all the mentioned flavonoids, the xo inhibitory activity exhibited by coriander seed extract can be assumed due to the binding of flavonoids to the xanthine binding site of the enzyme.

Identification of bioactive compounds present in coriander seeds using high performance thin layer chromatographic technique

High performance thin layer chromatography carried out for ethyl acetate fraction indicated very interesting information by means of development of spots in the chromatograms revealing the presence of many phenolic compounds, out of which quercetin and rutin could be identified and quantified, rutin (0.0066%) being predominant in the fraction followed by quercetin (0.0051%) (Figures 7, 8 and 9).



Figure 7: HPTLC finger print profile of ethylacetate fraction of coriander (*Coriandrum sativum* L.) seeds and the standard quercetin







Figure 9: Quercetin and rutin identified in the ethyl acetate fraction of coriander (*Coriandrum sativum* L.) seeds

Conclusions

Ethyl acetate fraction of coriander seeds is rich in bioactive especially phenolic compounds and therefore, coriander seeds are potent antioxidants that can interact with a wide range of oxidative species directly responsible for oxidative damage. The radical scavenging, anti-peroxidative and antiinflammatory activities of coriander seed extract are attributed to the phenolic compounds and the other bioactive compounds present therein, confirmed by qualitative and quantitative analysis. The seeds can be useful drugs to improve the health status of its users by virtue of a number of bioactive compounds, *viz*. polyphenols, flavonoids, tannins, steroids, terpenoids and saponins. More research is warranted to explore the wonderful therapeutic properties of medicinally less exploited coriander seeds.

Conflict of interest

We declare that we have no conflict of interest.

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