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# Antioxidant activity in extracts from coriander

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**Abstract**

Extracts of different polarity from leaves and seeds of coriander (*Coriandrum sativum*) and coriander oil were investigated for their antioxidant activity. Three different bioassays were used, namely scavenging of the diphenylpicrylhydrazyl (DPPH) radical method, inhibition of 15-lipoxygenase (15-LO) and inhibition of $Fe^{2+}$ induced porcine brain phospholipid peroxidation. Total phenolic content was quantified as well. Positive correlations were found between total phenolic content in the extracts and antioxidant activity. Coriander leaves showed stronger antioxidant activity than the seeds, and in both parts of coriander, the ethyl acetate extract contributed to the strongest activity. In conclusion, addition of coriander to food will increase the antioxidant content and may have potential as a natural antioxidant and thus inhibit unwanted oxidation processes.


*Keywords:* Coriander; *Coriandrum sativum*; Antioxidant; DPPH; 15-Lipoxygenase; Total phenolics; Radical-scavenging

## 1. Introduction

Coriander (*Coriandrum sativum* L.; Umbelliferae) is widely distributed and mainly cultivated for the seeds. The seeds contain an essential oil (up to 1%) and the monoterpenoid, linalool, is the main component (Wichtl, 1994). Coriander seed is a popular spice and finely ground seed is a major ingredient of curry powder. The seeds are mainly responsible for the medical use of coriander and have been used as a drug for indigestion, against worms, rheumatism and pain in the joints (Wichtl, 1994). Recent studies have also demonstrated hypoglycaemic action and effects on carbohydrate metabolism (Chithra & Leelamma, 2000; Gray & Flatt, 1999). Volatile components in essential oil, from both seeds and leaves, have been reported to inhibit growth of a range of micro-organisms (Delaquis, Stanich, Girard, & Mazza, 2002), and inhibition of lipid peroxidation is reported as well (Anilakumar, Nagaraj, & Santhanam, 2001; Tanabe, Yoshida, & Tomita, 2002).

It is well known that herbs and spices possess antioxidant activity (Madsen & Bertelsen, 1995; Schwarz et al., 2001; Tanabe et al., 2002), and caffeic acid derivates, flavonoids and terpenoids are suggested to be responsible for this effect (Madsen & Bertelsen, 1995). During recent years consumers have been more concerned about the addition of synthetic additives to food and the two most commonly used antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), have shown DNA damage induction (Sasaki et al., 2002). Therefore, there is an increasing interest in natural food additives, such as spices or spice extracts, which can function as natural antioxidants besides seasoning the food. Selection of a suitable extraction procedure can increase the antioxidant concentration relative to the plant material, and differences in antioxidant activity between the extracts indicate the polarity of the compounds mediating antioxidant effect.

Several analytical methods have been developed to determine the antioxidant capacity of natural substances in vitro. They can be categorized into two groups: (i) assays for radical-scavenging ability and (ii) assays for lipid oxidation inhibitory effect. However, the total antioxidant activities of plant extracts cannot be evaluated by using one single method, due to the complex

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composition of phytochemicals as well as of oxidative processes. Therefore, the use of at least two methods should be employed in order to evaluate the total antioxidant activity (Böhm, Schlesier, Harwat, & Bitsch, 2001). The aim of our study was to investigate the antioxidant activity of extracts of different polarity from both seeds and leaves of coriander. According to the recommendations, the antioxidant effects in three different bioassays were studied, besides determination of total phenolics.

## 2. Materials and methods

### 2.1. Materials

Coriander seeds (1.7 mm) and coriander essential oil were purchased from Norsk Medisinaldepot (Oslo, Norway), and fresh coriander leaves were obtained from a local grocery. Diphenylpicrylhydrazyl (DPPH) radical, linoleic acid and linalool (97%) were from Sigma and 15-lipoxygenase 15-LO from soybeans was from Fluka. All other reagents were of the highest purity available.

### 2.2. Extraction

Coriander seeds (2 kg) were extracted with $4 \times 5$ l dichloromethane at room temperature for 24 h prior to removal of the solvent in vacuo, yielding 350.6 g dichloromethane extract. The plant residue was further extracted similarly with $6 \times 5$ l aqueous ethanol (80%) for 24 h and the combined ethanol extracts were taken to dryness, yielding 102.5 g of crude extract. The crude extract was suspended in 0.5 l distilled water and extracted successively with $6 \times 0.5$ l of ethyl acetate and $5 \times 0.5$ l of *n*-butanol. After removal of solvents in vacuo, yields of 13.0 g (ethyl acetate extract) and 25.6 g (butanol extract) were obtained.

Coriander leaves (417 g) were finely chopped before extraction with $3 \times 4$ l aqueous ethanol (80%) for 24 h. After removal of the solvent in vacuo, the crude extract (17.3 g) was suspended in 0.2 l distilled water and extracted with 0.1 l portions of diethyl ether, ethyl acetate and *n*-butanol until the extracts were nearly colourless. Solvents were removed in vacuo, and yields of 5.6, 0.95 and 4.4 g, respectively, were obtained.

### 2.3. Total phenolic content

The total phenolic content was determined by the Folin–Ciocalteu (FC) method (Singleton & Rossi, 1965) and expressed as grammes of gallic acid equivalents per 100 g plant extract. Distilled water (3.16 ml) was mixed with a DMSO solution of the test compound (40 μl). Then, 200 μl of FC reagent was added. After 5 min, 600 μl of 20% sodium carbonate solution was added and the solutions were mixed again. The solutions were left at room temperature for 2 h. Then the absorption of the developed blue colour was determined at 765 nm, using a Shimadzu 160A UV-spectrophotometer (Shimadzu, Kyoto, Japan).

### 2.4. Antioxidant assays

#### 2.4.1. DPPH radical-scavenging

For testing radical-scavenging activity, reaction with the DPPH radical was carried out as previously described (Malterud, Farbrot, Huse, & Sund, 1993). Briefly, an aliquot of test compound (0.05 ml) (dissolved in DMSO) was mixed with a solution of DPPH in methanol ($A_{517} = 1.0$; 2.95 ml) and the UV absorbance at 517 nm was measured for 5 min. Percent radical-scavenging was calculated as $100 \times (A_{\text{start}} - A_{\text{end}})/(A_{\text{start}})$, where $A_{\text{start}}$ is the absorbance before addition of test compound and $A_{\text{end}}$ is the absorbance value after 5 min of reaction time.

#### 2.4.2. Inhibition of 15-LO

15-LO from soybeans was used for peroxidation of linoleic acid, and inhibition was carried out as described by Lyckander and Malterud (Lyckander & Malterud, 1992). To a solution of linoleic acid (final concentration 134 μM) in borate buffer (0.2 M, pH 9.00, 2.90 ml) was added 50 μl of test substance dissolved in DMSO or (for blanks) DMSO alone was added. A solution of 15-LO in 50 μl borate buffer (10,000 U/ml) was added, and the increase in absorbance at 234 nm for 30–90 s was measured. The value for % inhibition of enzyme activity was calculated as $100 \times [(\Delta A_1/\Delta t) - (A_2/\Delta t)/(\Delta A_1/\Delta t)]$, where $\Delta A_1/\Delta t$ and $\Delta A_2/\Delta t$ are values for increase in $A_{234}$ for sample without test substance and with test substance, respectively.

#### 2.4.3. Inhibition of phospholipid peroxidation

Inhibition of $Fe^{2+}$-induced peroxidation of phospholipids was carried out in a slight variation of a previously described method (Mathisen, Diallo, Andersen, & Malterud, 2002) using porcine brain as a source of phospholipids. In the assay, 50 μl of the phospholipid solution (25 mg/ml) in a mixture of chloroform and methanol (2:1) was mixed with 0.5 ml distilled water and 0.2 ml of DMSO (blanks), with 0.5 ml $FeSO_4$ (10 mM) and 0.2 ml DMSO (oxidant samples), or with 0.5 ml $FeSO_4$ solution and 0.2 ml of a DMSO solution of test compound. After incubation at 37 °C for 30 min, 0.5 ml thiobarbituric acid (1% aqueous suspension) and 4 ml trichloroacetic acid (10% aqueous solution) were added, and the mixture was heated on a boiling water bath for 15 min. After cooling on ice and centrifugation for 5 min, the absorbance at 532 nm was measured. The antioxidant activity was calculated as $100 \times (A_{\text{ox}} - A_{\text{test}})/(A_{\text{ox}} - A_{\text{blank}})$.

### 2.5. Statistics

Samples were assayed in triplicate and results are given as averages ± SD. Student's $t$ test was used for the statistical evaluation and $P < 0.05$ was considered statistically significant.

## 3. Results and discussion

### 3.1. Extraction

Initial experiments showed that the seeds contained large amounts of lipids, in contrast to the leaves. Therefore, the seeds were subjected to defatting with dichloromethane prior to extraction with aqueous ethanol. The dichloromethane extract contained only fat, and was inactive in assays for radical-scavenging and lipid peroxidation. In view of the inactivity of the dichloromethane extract, the ethanol extracts of the seeds and leaves can be used for comparison of activity.

### 3.2. Total phenolic content

The distribution of phenolic compounds in coriander demonstrated that the ethyl acetate extracts from both seeds and leaves contained highest amounts, 1.9 and 5.5 g gallic acid equivalents (GAE) per 100 g extract, respectively. As shown in Table 1, in all extracts, the contents of phenolics were higher in the leaves than in the seeds.

The FC method is actually not an antioxidant test but instead an assay for the quantity of oxidizable substances, i.e., phenolic compounds. Correlations between the content of phenolic compounds and antioxidant activity are described (Jiménez-Escrig, Jiménez-Jiménez, Pulido, & Saura-Calixto, 2001; Nuutila, Puupponen-Pimiä, Aarni, & Oksman-Caldentey, 2003; Parejo et al., 2002). The response order of the extracts was found to be significantly correlated with both DPPH radical-scavenging ($P < 0.025$) and inhibition of 15-LO peroxidation ($P < 0.001$) (Fig. 1), and may therefore be useful as an assay for detecting antioxidants in coriander.

Table 1
Total phenolic content in extracts from coriander and coriander oil

| | | GAE ± SD |
|---|---|---|
| Coriander seeds | | |
| | EtOH extract | 0.15 ± 0.01 |
| | $CH_2Cl_2$ extract | 0.09 ± 0.01 |
| | EtOAc extract | 1.89 ± 0.08 |
| | BuOH extract | 1.16 ± 0.01 |
| | Aqueous residue | 0.43 ± 0.00 |
| Coriander leaves | | |
| | EtOH extract | 0.36 ± 0.03 |
| | $Et_2O$ extract | 1.89 ± 0.07 |
| | EtOAc extract | 5.45 ± 0.09 |
| | BuOH extract | 1.42 ± 0.02 |
| | Aqueous residue | 1.06 ± 0.20 |
| Coriander oil | | 0.14 ± 0.01 |

Values are means of triplicates ± standard deviation, and expressed as g gallic acid equivalents (GAE)/100 g plant material.

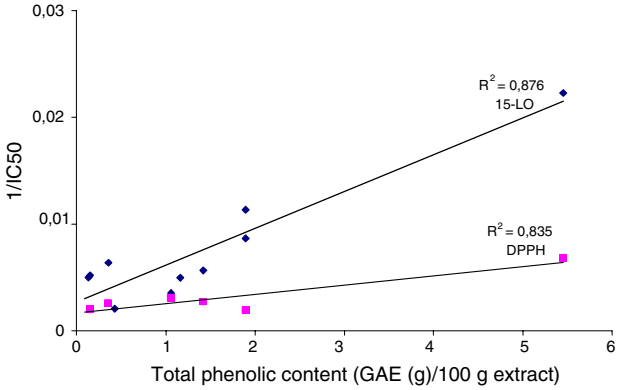


Fig. 1. Correlation between the activity (represented by $1/IC_{50}$) of the antioxidant tests and the concentration of total phenolic compounds.

### 3.3. Antioxidant assays

#### 3.3.1. DPPH radical-scavenging

The ethanol extracts from both leaves and seeds showed a concentration-dependent DPPH scavenging activity with $IC_{50}$ values of 389 ± 5 and 510 ± 12 μg/ml respectively. As shown in Fig. 2, the ethyl acetate extract from coriander leaves showed most potent activity ($IC_{50}$ value of 147 ± 3 μg/ml) and indicate that compounds with strongest radical-scavenging capacity are of medium polarity. Both coriander oil and the lipophilic extracts (coriander seed dichloromethane and coriander leaves diethyl ether extracts) were inactive in this assay, this may be explained by lack of hydrogen-donating capacity. Essential oil from coriander seeds has previously been investigated for radical-scavenging activity and it was found that it possessed weak DPPH scavenging activity (Puertas-Mejía, Hillebrand, Stashenko, & Winterhalter, 2002). In contrast, when using another antioxidant assay (TBARS), coriander oil demonstrated high antioxidant activity (Baratta, Dorman, Deans, Biondi, & Ruberto, 1998). To our knowledge, DPPH scavenging activity of coriander leaves have not been reported previously. Numerous plant products are reported to have DPPH scavenging effect, and flavonoids, coumarins, phenolic acids and terpenoids in coriander may probably contribute to this effect.

#### 3.3.2. Inhibition of 15-LO

Both ethanol extracts showed concentration-dependent effects towards 15-LO, and the leaves had higher activity than the seeds, with $IC_{50}$ values of 157 ± 9 and 193 ± 11 μg/ml, respectively (Fig. 2). The highest inhibitory activity toward 15-LO was observed in the ethyl

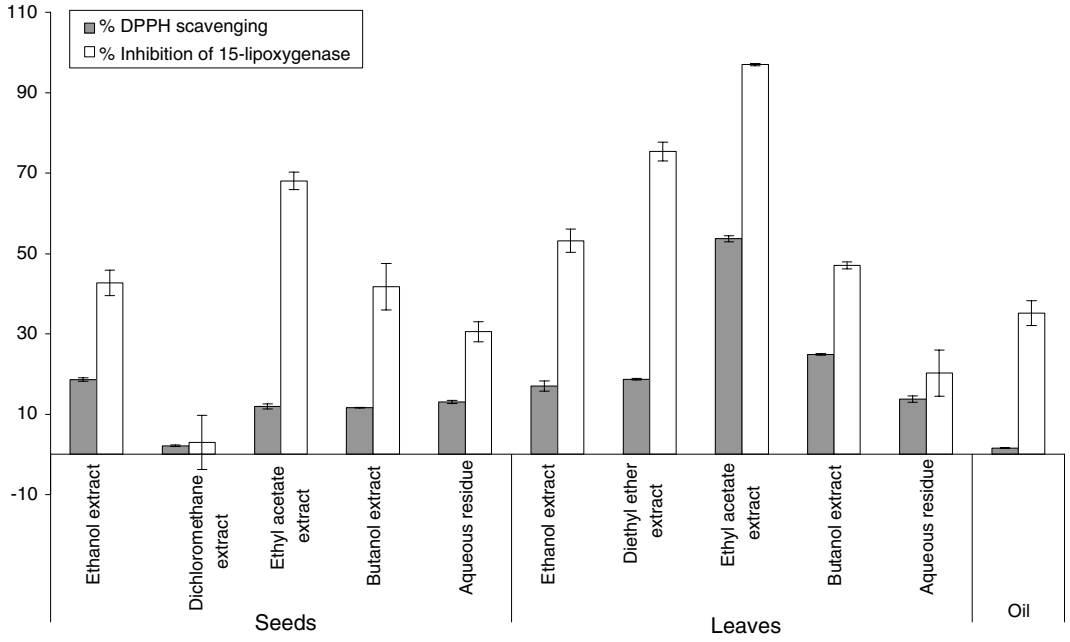


Fig. 2. Antioxidant activity of extracts (167 μg/ml) from seeds and leaves of coriander and coriander essential oil, measured as % scavenging of DPPH radical (grey) and % inhibition of 15-LO (blank). Each value represents mean ± SD of three experiments.

acetate extract from coriander leaves ($IC_{50} = 45 \pm 2$ μg/ml), and the diethyl ether extract from leaves also showed relatively high activity ($IC_{50} = 88 \pm 5$ μg/ml). Essential oil from coriander seeds showed weak inhibitory activity towards 15-LO ($IC_{50} = 199 \pm 11$ μg/ml), but its main component, linalool, was inactive as a 15-LO inhibitor ($IC_{50} > 667$ μg/ml). The identification of 15-LO inhibitors in coriander oil should be investigated in further studies. There seems to be a trend that lipophilic extracts contribute stronger activity in the 15-LO assay than the DPPH assay; thus the involvement of proton donation from the active compounds may be of less importance for 15-LO inhibition than is DPPH radical-scavenging.

In the literature, natural compounds reported as 15-LO inhibitors comprise, e.g., flavonoids (Lyckander & Malterud, 1992; Malterud & Rydland, 2000) and terpenoids (Amagata et al., 2003; Carroll, Jonsson, Ebel, Hartman, Holman, & Crews, 2001). Flavonoids and triterpenoids have also been reported from coriander leaves and seeds (Justesen & Knuthsen, 2001; Kunzemann & Herrmann, 1977; Naik, Namboori, & Merchanti, 1983), and may be involved in the observed 15-LO inhibition.

### 3.3.3. Inhibition of phospholipid peroxidation

With the phospholipid peroxidation method, the coriander extracts showed no inhibitory activity. We have previously observed that this assay is somewhat selective for lipophilic antioxidants (Mathisen et al., 2002). This may explain the different results obtained in the radical-scavenging assay from the $Fe^{2+}$/phospholipid assay. The "polar paradox", discussed by Porter, Black, & Drolet (1989), may also contribute to the difference in activities between these assay systems. Thus, these findings support the recommendation to use at least two methods when studying antioxidant activity.

## 4. Conclusion

In this study we have shown that both seeds and leaves from coriander have concentration-dependent inhibitory activity towards 15-LO and radical-scavenging properties. However, the effects are more potent in extracts from leaves than in seeds from coriander and it seems that compounds of medium polarity are most potent, even if their total antioxidant contribution in the plant is small. We have also shown a correlation between total phenolic content and antioxidant effect; thus a screening of phenolic content in coriander extracts will probably indicate the presence of compounds with antioxidant activity.

Our results indicate that inclusion of both seeds and leaves from coriander in the cuisine will increase the content of antioxidants, and thus probably prevent oxidative deterioration of food. It seems that several different compounds mediate antioxidant activity. However, it is uncertain if the quantity of spices in the diet is enough to have an influence on the antioxidant defence of the body.

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