ANTIOXIDANT POTENTIAL IN SEEDS OF CORIANDRUM SATIVUM: AN IN VITRO STUDY

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Abstract: In recent years, natural antioxidants in food and other biological materials have drawn considerable concern due to their presumed safety, nutritional and therapeutic value that offers protection against a range of non-communicable diseases and ageing. The present study focuses on evaluation of Coriandrum sativum (Coriander) varieties for their antioxygenic potential using different in vitro free radical scavenging models. The methanolic extracts of local and simco varieties of coriander were found to be appreciably effective in scavenging hydroxyl radical generated by Fenton reaction ($EC_{50} = 38$ and $18\,\mu g/ml$), super oxide radical generated by photoreduction of riboflavin ($EC_{50} = 553.50$ and $668.67\,\mu g/ml$), and nitric oxide radical generated in vitro from sodium nitroprusside ($EC_{50} = 180$ and $227\,\mu g/ml$) in a concentration dependent manner. In contrast, methanol: hexane extracts of both the varieties of coriander were found to be ineffective in quenching hydroxyl radical and revealed only moderate activity in quenching super oxide radical ($EC_{50} = 712$ and $832\,\mu g/ml$) and nitric oxide radical ($EC_{50} = 569$ and $703\,\mu g/ml$). However, hexane extracts exhibited no appreciable affects at either of the concentrations in all the three models. Instead, it showed pro-oxidant activity at higher concentrations. Above mentioned in vitro models proved Local variety to possess better antioxygenic potential. Likewise, the inhibition of in vitro linoleic acid peroxidation for longer period of incubation by methanolic extract (superior to ascorbic acid, a standard antioxidant) and lipid peroxidation in rat liver homogenate ($EC_{50} = 383\mu g/ml$) also indicated good antioxygenic potential of Local variety of coriander.

Key words: Coriander, Antioxidant activity, oxidative stress

INTRODUCTION

Oxidative stress is believed to play an important role in pathogenesis of ageing, inflammation and cancer [1]. Free radicals have also been implicated in etiology of various age related diseases such as atherosclerosis, asthma, stroke, vasospasms, liver damage and Alzheimer’s disease etc [2]. Partially reduced oxygen species such as superoxide anion radical ($O_2^-\cdot$), hydroxyl radical (OH), nitric oxide, hydrogen peroxide, peroxynitrite, hypochlorous acid etc which are collectively known as reactive oxygen species (ROS) can result in cell membrane disintegration by reacting with membrane proteins or lipids [3]. Moreover these can cause DNA mutation which further initiate or propagate development of many diseases [4]. Antioxidant status plays a pivotal role by inhibiting or delaying the oxidizing chain reaction to minimize oxidative damage which consequently prevents pathological changes [5]. In recent years, the use of natural antioxidants present in food and other biological materials has attracted considerable interest due to their presumed safety, nutritional and therapeutic value [6]. Plant foods contain a large number of phyto-nutrients that have the potential for offering a good degree of protection against a range of non-communicable diseases, like cancer, cardiovascular disease and cataract [7].
Coriandrum sativum (CS), is an umbelliferous annual plant. Different parts of the plant are used for medicinal purposes such as dyseptic complaints and loss of appetite [10]. Preliminary reports have indicated the antioxidant potential of CS in different in vitro models. However, the antioxidant potential is highly variable with respect to the variety, cultivation practices and location [11]. Efforts to gain extensive knowledge regarding power of antioxidants from this plant has increased [12]. Previous phytochemical investigations on coriander by various authors demonstrated the presence of terpenoids and flavonoids and related polyphenols in the extracts of coriander [13,14]. Hence, the present study has been undertaken to investigate the antioxidant activity of seeds of two varieties (Local and Simco) of CS.

MATERIALS AND METHODS

Two varieties of CS i.e. Local and Simco, were obtained from Department of Agronomy and Agrometeorology, Punjab Agricultural University, Ludhiana. About 100g of shade dried coriander seeds were ground to a fine powder and were exhaustively extracted with solvents of varying polarities such as hexane, methanol and hexane-methanol mixture (3:7) by refluxing at 60°C for 12 hours. After filtration, the residues were re-extracted twice under same conditions. Solvent was removed from the combined filtrates under vacuum at 45°C in evaporator. The yield of crude extracts was determined gravimetrically. The extracts were then stored in a desiccators until further use. The crude extracts were redissolved in distilled water when needed. For the evaluation of antioxidant potential, the extracts obtained from solvents of varying polarity were tested in different in vitro models (hydroxyl radical, superoxide radical and nitric oxide radical scavenging potential).

Determination of hydroxyl radical scavenging activity- Hydroxyl radical scavenging activity was determined by deoxyribose degradation method [15] on the basis of study of competition between deoxyribose and test compounds for hydroxyl radicals produced by Fe²⁺-ascorbate-EDTA-H₂O₂ system (Fenton reaction). The hydroxyl radicals attack deoxyribose that eventually results in formation of thiobarbituric acid reacting substances (TBARS) method [16]. The reaction mixture consisted of 0.1ml of deoxyribose (2.8 mM), 0.1ml of FeCl₃ (0.1 mM), 0.1ml of EDTA (0.1 mM), 0.1ml of ascorbic acid (0.1 mM), 0.1ml of H₂O₂ (1 mM), phosphate buffer (20 mM, pH 7.4) and various concentrations of coriander extracts in a final volume of 1 ml. The reaction mixture was incubated for 1 h in a water bath at 37°C. Then 3ml of thiobarbituric acid (TBA) was added followed by vigorous shaking and incubated for 1h in a boiling water bath. The % inhibition was determined by comparing the absorbance values at 532 nm of test and control.

Determination of superoxide radical scavenging activity- Superoxide radical scavenging activity was determined by inhibition of light induced (photoreduction) superoxide radical generation by riboflavin and subsequent reduction of NBT [17]. The reaction mixture consisted of 0.1 ml of EDTA (6.6 mM) containing 3 mg NaCN, 0.1 ml of riboflavin (2mM), 0.1 ml of NBT (50mM), various concentrations of coriander extracts and phosphate buffer in a final volume of 3 ml. The tubes were uniformly illuminated with incandescent lamp for 15 min and the optical density was recorded at 530 nm before and after the illumination. The % inhibition of super-oxide generation was measured by comparing the absorbance values of test and control compounds.

Determination of nitric oxide radical scavenging activity- Nitric oxide radical scavenging activity was determined by detecting the inhibition of nitric oxide generation. Aqueous solution of sodium nitroprusside spontaneously generates nitric oxide (NO‘) at physiological pH, which interacts with oxygen to form nitrite ions. These nitrite ions were measured colorimetrically, by their diazotization with sulphanilamide and its subsequent coupling to naphthylethylene diamine [18]. Reaction mixture consisted of 1ml of sodium nitroprusside (10 mM), various concentrations of coriander extracts and phosphate buffered saline (PBS) to make a total volume of 3 ml. The reaction mixture was incubated at 25°C for 150 min. After incubation, 0.5 ml of incubation mixture was drawn and to this 0.5 ml of Griess reagent (1% sulphamylamide, 2% phosphoric acid and 0.1% naphthyl-ethylene-diamine dihydrochloride) was added. The absorbance of chromophore formed was read at 546 nm after making the final volume of 4 ml with distilled water. The % inhibition of nitric oxide generation was measured by comparing the absorbance value of test and control.

Determination of Lipid peroxidation inhibiting activity using linoleic acid emulsion- The antioxidant activity of coriander was determined by detecting the ability of extract to inhibit H₂O₂ induced lipid peroxidation of linoleic acid. Different concentra-
tions of extracts of coriander were mixed with 2.5 ml of linoleic acid emulsion (pH 7.0) in phosphate buffer (0.2 M, pH 7.0). The reaction mixture was incubated at 37°C. Aliquots (10 ml) were taken at different time intervals during incubation. The degree of oxidation was measured according to the thiocyanate method [19], by sequentially adding ethanol (4.7 ml, 75%), ammonium thiocyanate (0.1 ml, 30%), sample solution (10 ml) and ferrous sulphate (0.1 ml, 0.02M in 3.5% HCl). The mixture was allowed to stand for 3 min and absorbance was recorded at 500 nm. Vitamin C was used as a positive control.

**In vitro lipid peroxidation of rat liver homogenate:** It was measured by Bishayee and Balasubramaniyam method [20]. Freshly excised rat liver was processed to get 10% rat liver homogenate prepared in cold phosphate buffered saline (pH 7.4) using a glass Teflon homogenizer and filtered to get a clear homogenate. The incubation mixture contained 0.5ml of 10% rat liver homogenate, 0.1ml of ferrous iron (0.5 mM), 0.1 ml of ADP (1.7 mM), 0.1ml of ascorbic acid (0.5 mM) and the final volume was made up to 1.5 ml with KCl (0.15 M). Mixture was incubated for 20 min at 37°C in presence and absence of different concentrations of coriander extracts. After incubation, 0.6 ml of reaction mixture was taken and treated with 0.2 ml sodium dodecyl sulphate (8.1%), 1.5 ml of thiobarbituric acid (0.8%) and 1.5 ml of acetic acid. The mixture (4 ml) was then kept in a boiling water bath for 1 h. After cooling, 1 ml of distilled water and 5 ml of a mixture of n-butanol and pyridine were added and shaken vigor-ously. Followed by centrifugation, the absorbance of the upper layer containing chromophore was read at 532 nm. The % inhibition of lipid peroxidation by the extract was determined by comparing the absorbance values of control and experimental tubes.

**EC<sub>50</sub> values:** The concentration of extracts required for 50% inhibition of free radical generation, were determined by plotting the % inhibition vs concentration of the extract. By considering, EC<sub>50</sub> values and the yield of the extracts obtained from both of the varieties of coriander, EC<sub>50</sub> equivalents/g of coriander seeds were calculated as EC<sub>50</sub> equivalents/g = Yield of extract (μg/g)/ EC<sub>50</sub> value (μg/ml).

**RESULTS AND DISCUSSION**

Yields of the extracts of both varieties of CS with different solvents were quite different. Local variety had 6.65% yield where as Simco variety yielded 5.60% in case of methanolic extracts. Likewise, for methanol:hexane extracts, Simco variety (7.19%) had better yield than Local variety (6.27%). Where as in case of the hexane extracts Local variety gave better yield (6.61%) than Simco variety (4.60%).

**Antioxidant activity of hexane and hexane-methanol extracts:** No appreciable effect of hexane extracts was seen at either of the concentrations in all the three models. Instead, the extract showed pro-oxidant activity at higher concentrations. This rule out the free radical scavenging potential of hexane extract. Methanol:hexane extracts were also found to be ineffective in scavenging of hydroxyl radical (Fig. 1A). However, the methanol:hexane extracts of both the varieties showed some superoxide radical and nitric oxide radical scavenging potential. The inhibitory potential of Local variety of coriander yielded better results than Simco variety in both the models. The concentrations needed for 50% inhibition (EC<sub>50</sub>) of superoxide production were found to be 712 and 832 mg/ml respectively (Fig. 1B) and EC<sub>50</sub> equivalents/g were 88 and 86 equivalents for Local and Simco varieties respectively. The concentrations needed for 50% inhibition of nitrite production, were 569 μg/ml for Local variety and 703 μg/ml for Simco variety (Fig. 1C). The EC<sub>50</sub> equivalents/g of CD worked out to be 110 and 102 respectively for both varieties.

**Antioxidant activity of methanolic extracts:** The methanolic extracts of CS were highly effective in scavenging free radicals in a concentration dependent manner in all the in vitro models used.

(a) **Hydroxy radical scavenging activity:** Concentrations of local and simco variety needed for 50% inhibition of hydroxyl radical generation were found to be 38 and 18 μg/ml respectively (Fig. 2A). EC<sub>50</sub> equivalents/g of coriander for hydroxyl radical scavenging potential was found to be 3628 and 1493 for Local variety and Simco variety respectively (Table 1).

(b) **Superoxide radical scavenging activity:** The methanolic extracts of coriander exhibited even better inhibition against superoxide radicals generated by photo reduction of riboflavin in concentration dependent manner. The inhibitory potential followed the same scenario where Local variety displayed the better potential than Simco variety and concentrations needed for 50% inhibition (EC<sub>50</sub>) were found to be 553.50 and 668.67 μg/ml respectively (Fig. 2b). The
Fig. 1: Effect of methanol:hexane extract of Local and Simco varieties of coriander on oxygen radical generation in vitro. (A) hydroxyl radical (B) superoxide radical (C) nitric oxide radical.

Fig. 2: Effect of methanolic extracts of Local and Simco variety of coriander on oxygen radical generation in vitro- (A) hydroxyl radical (B) superoxide radical (C) nitric oxide radical.
Table 1: Antioxidant activity of methanolic extract of both the varieties of coriander - EC₅₀ equivalents/g of coriander seeds.

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>MDA formed (nmoles/h/ml of tissue homogenate)</th>
<th>% Protection</th>
</tr>
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<tbody>
<tr>
<td>20</td>
<td>55.24 ± 2.23</td>
<td>5.99 ± 5.79</td>
</tr>
<tr>
<td>50</td>
<td>51.67 ± 3.69</td>
<td>12.33 ± 3.07</td>
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<tr>
<td>100</td>
<td>45.2 ± 2.6</td>
<td>26.48 ± 2.83</td>
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<td>200</td>
<td>33.78 ± 2.03</td>
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<tr>
<td>500</td>
<td>27.025 ± 1.49</td>
<td>54.04 ± 1.55</td>
</tr>
<tr>
<td>1000</td>
<td>19.474 ± 1.12</td>
<td>65.57 ± 2.52</td>
</tr>
<tr>
<td>Control</td>
<td>58.821 ± 2.98</td>
<td>-</td>
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ROS scavenged by in vitro model | EC₅₀ equivalents/g of coriander seeds
--- | ---
Hydroxyl radical | 3627.93 | 1493.33
Superoxide radical | 120.14 | 83.75
Nitric oxide radical | 370.41 | 247.06

EC₅₀ equivalents/g of coriander computed were 120 and 84 respectively for Local variety and Simco variety (Table 1).

(c) Nitric oxide radical scavenging activity: Inhibition potential of methanolic extracts of both the varieties of coriander against nitric oxide radical generation proved better than that of hexane and methanol-hexane extracts. Local variety revealed better inhibitory potential than Simco variety rendering EC₅₀ values of 180 and 227 µg/ml respectively (Fig. 2C). The EC₅₀ equivalents/g of coriander, were calculated to be 370 and 247 for Local and Simco varieties of coriander respectively (Table 1). The concentration of ascorbic acid needed for 50% inhibition was found to be much higher i.e. 2700 µg/ml.

These results highlights that Local variety exhibited better inhibitory potential than Simco for all three in vitro models (hydroxyl radical scavenging potential, super oxide radical scavenging and nitric oxide radical scavenging potential). Different varieties of the same herb are known to show relative antioxidant efficiencies [11]. The EC₅₀ values obtained in the present study were different in each assay which could be due to difference in their sensitivity. Methanolic extracts exhibited most potent in vitro antioxidant activity with high percentage inhibition as compared to other solvents (21). Since only the methanolic extracts were effective anti-oxidants it appears that the antioxidant potential of coriander could be attributed to its high flavonoid content. From in vitro evaluation of antioxidant activity of two varieties of coriander, the Local variety which proved to be potent in in vitro radical scavenging studies was selected for further evaluation of its antioxidigenic potential using models more close to in vivo system i.e. oxidation of linoleic acid and liver homogenates.

Inhibition of linoleic acid peroxidation: The methanolic extract of coriander exhibited inhibition of the peroxidation of linoleic acid in a concentration dependent manner (Table 2). The coriander extract was highly effective in preventing the peroxidation of linoleic acid even up to 48h at higher concentrations. On comparison of coriander extract with standard antioxidant ascorbic acid, the antioxidant activity in this system was found to be far better in coriander. Ascorbic acid gained the maximum inhibition of 75 % till 12h where as coriander exhibited maximum inhibition of 80 % till 48h of incubation period.

The functional methods that simulate oxidative reactions similar to those occurring in vivo explore their protective effects against oxidative reactions [22-24]. As relatively polar solvent extract showed better antioxidant property the presence of various...
flavonoids in herbal extracts might have been involved in inhibition of peroxidation [25]. It has been reported that the total phenolic contents or probably some other factors in various herbs play major role in the antioxidant potential against linoleic acid peroxidation system [26]. Lipid peroxidation, caused by free radicals leading to oxidative destruction of PUFA a constituent of cellular membranes produces toxic and reactive aldehyde metabolite i.e. malondialdehyde (MDA) which is most commonly measured as TBARS [27].

**Inhibition of lipid peroxidation in rat liver homogenate:** Addition of herbal methanolic extract of local variety of coriander at various concentrations was found to inhibit peroxides generated by Fe²⁺-ADP-ascorbate in rat liver homogenate in a dose dependent manner. The concentration of local variety of coriander needed for 50% inhibition was found to be 383µg/ml. The protection of membrane lipids by the methanolic extracts is evidenced by the MDA formed and % protection in *in vitro* lipid peroxidation.

The present study indicated a strong antioxidant action of methanolic extracts of both the varieties of coriander as evidenced by low EC₅₀ values. However, the extracts of coriander prepared using hexane as solvent did not show any antioxidant activity. Instead, a pro-oxidant activity was exhibited at higher concentrations. The methanol : hexane extracts exhibited mild to moderate antioxidant activity only at some critical concentrations. Moreover, the quantity of the methanolic extracts needed for the *in vitro* inhibition of oxygen radicals such as hydroxyl radicals, superoxide radicals, nitric oxide radicals and lipid peroxides were relatively low and superior to methanol-hexane extracts. As the methanolic extract of local variety of coriander is been highly enriched in antioxidant compounds, it can be exploited to prepare effective anti-oxidant preparations for food supplements or nutraceuticals.

**REFERENCES**