Isolation and simultaneous detection of flavonoids in the methanolic and ethanolic extracts of *Coriandrum sativum* L. seeds by RP-HPLC

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Abstract

Reversed-phase high performance liquid chromatography (RP-HPLC) method with UV/VIS detection was established for the separation and identification of flavonoids in the methanolic and ethanolic extracts of coriander (*Coriandrum sativum* L.) seeds. Separation was achieved using a column RP-C<sub>18</sub> VARIAN Pursuit XPs with dimensions 250 x 4.6mm using a mobile phase of formic acid and acetonitrile gradient. Rutin, quercetin, chlorogenic acid and caffeic acid were identified and quantified in the multi-component methanolic and ethanolic extracts by comparing with the respective standards using the method of RP-HPLC. The methanolic extract produced sharp peaks in comparison to the ethanolic extract. Chlorogenic acid was predominant in methanolic extract followed by rutin, caffeic acid and quercetin while rutin was predominant in ethanolic extract followed by chlorogenic acid, caffeic acid and quercetin. Though similar compounds were identified in both the extracts, the concentration and purity of the compounds were superior in the methanolic extract as compared with that of the ethanolic extract. Besides, chromatograms of ethanolic extracts had peak tailing which indicates that the bio-active principles are extracted better in methanol.

Key words: RP-HPLC, *Coriandrum sativum* L., flavonoids, rutin, quercetin, chlorogenic acid, caffeic acid.

Introduction

Coriander (*Coriandrum sativum* L.) is a culinary plant from the family Umbelliferae, which is extensively cultivated in India, Russia, central Europe, Asia and Middle East. The dried fruits are extensively employed as a condiment, especially for flavoring sauces, meat products, bakery and confectionery items (Ravi et al., 2007). Coriander seeds contain an essential oil (up to 1%) and the monoterpenoid, linalool, is the main component. Coriander seed is a popular spice and finely ground seed is a major ingredient of curry powder. The seeds are mainly responsible for the medicinal use of coriander and have been used as a drug for indigestion, against worms, rheumatism and pain in the joints (Wichtl, 1994). In the folk medicine, the seeds of coriander are used as an aromatic, carminative, stomachic, antispasmodic and against gastrointestinal complaints such as dyspepsia, flatulence and gastralgia. The seeds are also used as an ingredient in the laxative preparations to prevent stomach griping (Nadakarni, 1976; Jain, 1991).

The volatile compounds are major components of spice and aromatic plants. They are isolated by steam distillation and received the name essential oils which exhibit diverse biological properties, including antioxidant activity (Lee and Shibamoto, 2001). Polyphenolic phytochemicals are ubiquitous in the plant kingdom. These important aromatic secondary metabolites of plants are consumed in significant amounts in daily life. The composition of polyphenolic phytochemicals is influenced by maturity, cultivar (Lee and Jaworski, 1987), cultural practices, geographic origin, climatic and storage conditions and processing procedures (Spanos and Wrolstad, 1990). Some phytochemicals are known as nutraceuticals, which provide health benefits because of their biological activities (Dillard and German, 2000). Research on phytochemicals has been driven in recent years by their beneficial health effects, including antioxidant, anticarcinogenic, and antimutagenic activities (Huang et al., 1992) and their ability to reduce the risk of coronary heart disease (Hertog et al., 1993).

Many of today’s synthetic drugs originate from the plant kingdom. Herbal drugs are proved as effective as synthetic drugs with lesser side effects (Balasubramanian et al., 2005). Coriander is one of a few savory plants, a potential source of phenolic compounds having biological activities. In Morocco, coriander has been documented as a traditional treatment for diabetes, indigestion, flatulence, insomnia, renal disorders and loss of appetite, and is a diuretic and all parts of the plant are edible, but the fresh leaves and the dried seeds are the most common parts used in cooking (Aissaoui et al., 2008). Due to the complexity of the natural mixtures of phenolic compounds of various plants it is rather difficult to elucidate their structure and assess the antioxidant and biological potentials. Indeed, the determination of individual flavonoid glycosides form plant extracts could prove to be a difficult task.

The aim of this work was to analyze and identify some of the phenolic compounds present in coriander (*Coriandrum sativum* L.) seeds by using RP-HPLC, a high-resolution chromatographic technique widely used for the simultaneous extraction and identification of phenolic compounds.
Materials and Methods

Apparatus

The chromatography separation was performed using a Shimadzu LC-20AD with a quaternary pump system. Auto injector or auto sampler (SIL-20ACHT) was used for 20 µl of sample injection. Separation was carried out at ambient temperature with a column (VARIAN Pursuit XPs- C\textsubscript{18}) dimension 250 x 4.6mm, S/N 436, protected by a guard column. The detector signal was recorded with a UV/VIS detector (SPD-20A).

Reagents

Formic acid, acetonitrile, methanol, ethanol, water and all the other reagents were of HPLC grade.

Plant material

Coriander seeds were purchased from local grocery, cleaned to be free from extraneous materials, shade dried and ground to a coarse powder using electric blender.

Preparation of sample extracts

The extraction was carried out by mixing 50g of seed powders in 250ml of methanol and ethanol separately. Both the solutions were stirred regularly for 15 days. The extracts were filtered and dried using flash evaporator. The residues after evaporation were weighed 15 days. The extracts were filtered and dried using flash evaporator. The residues after evaporation were weighed.

Preparation of standard solutions

Rutin and quercetin: Standard stock solutions of two flavonoids, rutin and quercetin were prepared in methanol and ethanol separately, at concentrations of 100ppm (10 mg of the standards were dissolved in 25ml methanol and ethanol in separate volumetric flasks, sonicated and volume made up to 25 ml with respective solvents to give 400ppm, 2.5ml of stock solution was taken and made up to 10ml with methanol and ethanol respectively to give concentrations of 100ppm).

Caffeic acid and chlorogenic acid: Standard solutions were prepared by dissolving 10mg of the standards in methanol and ethanol separately in 25ml volumetric flasks, sonicated and volume was made up to 25 ml with respective solvents to give concentration of 400ppm.

Procedure

RP-HPLC with C\textsubscript{18} columns is the most popular technique for the analysis of polyphenols of the different foods. A UV-vis multiwavelength detector (SPD-20A) was used because all phenolic compounds show intense absorption in the UV region of the spectrum. This method used for the separation of caffeic acid and chlorogenic acid (320nm), rutin and quercetin (370nm) included mobile phase 0.5% formic acid: Acetonitrile (ACN)(70:30) at a flow rate 0.9ml/min; column (VARIAN Pursuit XPs- C\textsubscript{18} dimension 250 x 4.6mm) at 40°C temperature. The polyphenols identification was based on the comparison of their retention times with those of the standard solutions (Sigma-Aldrich).

Results and Discussion

Free radicals and other reactive species are thought to play an important role in many human diseases. The plants have been regarded as having considerable health benefits, due to their main antioxidant compounds viz. phenolics which effectively neutralize or scavenge the free radicals. Flavonoids, a group of phenolics almost universal pigments of plants, are important parts of the human diet and considered as active principles of many medical plants. There are a few reports on flavonoid constituents of Coriandrum sativum L. The literature points out that some activities can be especially related to these flavonoids i.e., antioxidant, anti-inflammatory, anticarcinogenic, antimutagenic antifulmic, antihepatotoxic and antiangiogenesis for quercetin, antiplatelet and vasodilatatory activities for luteolin (Halliwell and Whitman 2004).

Phytochemicals, especially phenolics in plant foods, are suggested to be the major bioactive compounds responsible for their health benefits, which can be attributed to the antioxidant and metal chelating abilities of phenolic compounds. Phenolics have been shown to be highly effective scavengers of most types of oxidizing molecules, including singlet oxygen and other free radicals produced by lipid peroxidation (Calgorotto et al., 2007). 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 phenolic compounds in plant materials is influenced by their chemical nature, the extraction method employed, sample 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 non-phenolic substances such as waxes, fats, terpenes and chlorophylls (Naczk and Shahidi, 2004).

Due to the complexity of the natural mixtures of phenolic compounds of various plants, it is rather difficult to elucidate their structure and assess the antioxidant and biological potentials. Indeed, the determination of individual flavonoid glycosides form plant extract could prove to be a difficult task. Hence, it was aimed in this work to isolate and identify some of the phenolic compounds present in the methanolic extract of coriander (Coriandrum sativum) seeds by using RP-HPLC which is a high-resolution chromatographic technique widely used for the simultaneous extraction and identification of some of the phenolic compounds.

In the course of optimization of the methods for separation and analysis of the flavonoid aglycones caffeic acid, chlorogenic acid, quercetin and rutin the in seeds of...
Coriandrum sativum L. through reversed-phase high performance liquid chromatography (RP-HPLC) with UV detection, different combinations of isocratic and gradient techniques and good resolution of the flavonoids was achieved. The flavonoids were identified by comparison with the chromatogram of the standard flavonoid compounds obtained under similar conditions. This method gave a quick analysis of the flavonoids present in the methanolic and ethanolic extracts of Coriandrum sativum L. seeds.

The retention time (RT) for the standards viz. methanolic caffeic acid, chlorogenic acid, quercetin and rutin were 4.033, 3.605, 11.638 and 3.711, (Fig 1,2,4 and 5) respectively while the retention time (RT) for the same standards prepared in ethanol were 4.048, 3.754, 11.637 and 2.602, (Fig 7,8,10 and 11) respectively and compounds in the methanolic (Fig. 3 and 6) and ethanolic (Fig. 9 and 12) extracts were identified by comparing their retention times with those of the standards.

The experimental data pertaining to retention times, area under standard peaks as well as sample peaks of various polyphenols in the methanolic and ethanolic extracts are presented in Table 1 and 2 respectively. All the four flavonoids were in higher concentration in the methanolic extract than those in the ethanolic extract (Table 1 and 2) indicating better extractability of the flavonoids in methanol. Methanolic extract had highest amount of chlorogenic acid while ethanolic extract had highest amount of rutin. The methanolic extract produced sharp peaks in comparison to the ethanolic extract. Chlorogenic acid was predominant in methanolic extract followed by rutin, caffeic acid and quercetin whereas rutin was predominant in ethanolic extract followed by chlorogenic acid, caffeic acid and quercetin. Though similar compounds were identified in both the extracts, the concentration and purity of the compounds were superior in the methanolic extract as compared with that of the ethanolic extract. Besides, chromatograms of ethanolic extracts had peak tailing which indicates that the bio-active principles are extracted better in methanol which could be due to variable nature and solubility of the compounds present in respective extracts.
Fig. 1 HPLC chromatogram of standard caffeic acid in methanol

Fig. 2 HPLC chromatogram of standard chlorogenic acid in methanol

Fig. 3 HPLC chromatogram of methanolic extract
Fig. 4 HPLC chromatogram of standard quercetin in methanol

Fig. 5 HPLC chromatogram of standard rutin in methanol

Fig. 6 HPLC chromatogram of methanolic extract
(Detection at 370 nm, peaks: R-rutin, Q-quercetin)
Fig. 7 HPLC chromatogram of standard standard caffeic acid in ethanol

Fig. 8 HPLC chromatogram of chlorogenic acid in ethanol

Fig. 9 HPLC chromatogram of ethanolic extract (Detection at 320nm, peaks: Ch-chlorogenic acid, Cf-caffeic acid)
Fig. 10 HPLC chromatogram of standard quercetin in ethanol

Fig. 11 HPLC chromatogram of standard rutin in ethanol

Fig. 12 HPLC chromatogram of ethanolic extract (Detection at 370nm, peaks: R-rutin, Q-quercetin)
Table 1

Retention time, peak area and concentration of the compounds in the methanolic extract of coriander seeds

<table>
<thead>
<tr>
<th>S. No</th>
<th>Compounds</th>
<th>Retention time</th>
<th>Peak area of the standard</th>
<th>Peak area of the compound in the sample</th>
<th>Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Caffeic acid</td>
<td>4.033</td>
<td>30271994</td>
<td>2537878</td>
<td>1.89</td>
</tr>
<tr>
<td>2</td>
<td>Chlorogenic acid</td>
<td>3.605</td>
<td>11466523</td>
<td>14420915</td>
<td>28.01</td>
</tr>
<tr>
<td>3</td>
<td>Quercetin</td>
<td>11.638</td>
<td>9280414</td>
<td>182395</td>
<td>0.11</td>
</tr>
<tr>
<td>4</td>
<td>Rutin</td>
<td>3.711</td>
<td>2307114</td>
<td>4960987</td>
<td>11.48</td>
</tr>
</tbody>
</table>

Table 2

Retention time, peak area and concentration of the compounds in the ethanolic extract of coriander seeds

<table>
<thead>
<tr>
<th>S. No</th>
<th>Compound</th>
<th>Retention time</th>
<th>Peak area of the standard</th>
<th>Peak area of the compound in the sample</th>
<th>Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Caffeic acid</td>
<td>4.048</td>
<td>35611226</td>
<td>912467</td>
<td>0.074</td>
</tr>
<tr>
<td>2</td>
<td>Chlorogenic acid</td>
<td>3.754</td>
<td>1688610</td>
<td>449472</td>
<td>0.45</td>
</tr>
<tr>
<td>3</td>
<td>Quercetin</td>
<td>11.637</td>
<td>9059782</td>
<td>223896</td>
<td>0.07</td>
</tr>
<tr>
<td>4</td>
<td>Rutin</td>
<td>2.602</td>
<td>1788621</td>
<td>856806</td>
<td>1.30</td>
</tr>
</tbody>
</table>

Conclusion

In the prepared multi-component extracts of coriander seeds, good amounts of rutin, quercetin, chlorogenic acid and caffeic acid were identified. The analysis revealed rutin to be predominant in both the extracts followed by quercetin, chlorogenic acid and caffeic acid. Though similar flavonoids were identified in both the extracts, the concentration and purity of all the compounds were superior in the methanolic extract as compared with that of the ethanolic extract. The chromatograms of extract prepared in ethanol showed additional peaks indicating the presence of some unidentified compounds released during the extraction in ethanol and identification of such compounds warrants further investigation.

References