International Journal of Basic and Applied Sciences Vol. 1 No. 1. 2012. 21-26
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Full Length Research Paper

Reverse phase HPLC for the detection of flavonoids in the ethanolic extract of *Coriandrum sativum* L. seeds

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ABSTRACT

Coriandrum sativum L., an important spice, occupies a prime position in flavoring substances. Seed spices contain variable amounts of protein, fat, carbohydrate, fiber, minerals and vitamins along with flavouring compounds. The aim of the study was to identify and quantify flavonoids in the ethanolic extract of coriander seeds by comparing with the standards using the method of RP-HPLC. Separation was achieved using a column RP-C_{18} VARIAN Pursuit XPs with dimensions 250 x 4.6mm using a mobile phase of formic acid and acetonitrile gradient. The analysis revealed the presence of caffeic acid, chlorogenic acid, quercitin and rutin while rutin being predominant in the extract followed by chlorogenic acid, caffeic acid and quercetin. The method is simple sensitive, reproducible and very suitable for the determination of flavonoids viz. caffeic acid, chlorogenic acid, quercitin and rutin in the ethanolic extract.

Key words: *Reversed-phase high performance liquid chromatography (RP-HPLC), Coriandrum sativum L., flavonoids, caffeic acid, chlorogenic acid, quercetin, rutin.*

INTRODUCTION

Herbal drugs are proved as effective as synthetic drugs with lesser or no side effects (Balasubramanian et al. 2005). Herbs and spices synthesize innumerable compounds in their system; hence, these are often reported as store house for bioactive compounds. Coriander (*Coriandrum sativum* L.) a culinary and medicinal plant of Umbelliferae family, is extensively cultivated in India, Russia, Central Europe, Asia and Middle East. The dried fruits (seeds) are extensively employed as a condiment, especially for flavoring sauces, meat products, bakery and confectionery items (Ravi et al. 2007). Coriander is widely distributed and mainly cultivated for the seeds which contain an essential oil (up to 1%) and the monoterpenoid- linalool, is the main component. The seeds are mainly responsible for the medicinal use of coriander and they have been used as a drug for indigestion, against worms, rheumatism and pain in the joints (Wichtl, 1994). In Morocco, coriander has been documented as a traditional treatment for diabetes, indigestion, flatulence, insomnia, renal disorders and loss of appetite and as a diuretic. 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).

Like other spices, coriander is available throughout the year providing a fragrant flavour that is reminiscent of both citrus peel and sage. The fruit of the coriander plant contains two seeds which, when dried, are the portions used as the dried spice. When ripe, the seeds are yellowish-brown in color with longitudinal ridges. Coriander is rich in beneficial phytonutrients, including *carvone, geraniol, limonene, borneol, camphor, elemol,* and *linalool.* Coriander's flavonoids include *quercetin, keampferol, rhamnetin and apigenin.* Plus, coriander contains active phenolic acid compounds (flavonoids, polyphenols) which are also known to inhibit free radicals generated in the cellar system. The study of antioxidants that are ubiquitously present in spices is gaining momentum in human health as these are easily absorbable in human system.

Polyphenolic phytochemicals are ubiquitous in the plant kingdom. **Table 1** shows structures of phenolic compounds (caffeic acid, chlorogenic acid, quercetin and rutin) present in *Coriandrum sativum* L. Phenolic compounds are important aromatic secondary metabolites of plants that are consumed in significant amounts in daily life. The composition of

polyphenolic phytochemicals is influenced by maturity, cultivar (Lee & Jaworski, 1987), cultural practices, geographic origin, climatic conditions, storage conditions and processing procedures (Spanos & Wroslad, 1990). Some phytochemicals are known as neutraceuticals which provide health benefits because of their biological activities (Dillard & German, 2000). In recent years, research on phytochemicals has been driven by their beneficial health effects, including antioxidant, anticarcinogenic and antimutagenic activities (Huang & Ferraro, 1992) and their ability to reduce the risk of coronary heart disease (Hertog et al. 1993). Coriander is one of a few savory plants, a potential source of phenolic compounds with biological activities.

Due to the complexity of the natural mixtures of phenolic compounds, it is rather difficult to elucidate their structure and assess the antioxidant and biological potentials. Indeed, the determination of individual flavonoid glycosides from 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 ethanolic extract of coriander seeds by using RP-HPLC which is a high-resolution chromatographic technique widely used for the isolation and identification of some of the phenolic compounds.

MATERIALS AND METHODS

Chemicals

Caffeic acid, chlorogenic acid, quercetin and rutin standards were obtained from Sigma-Aldrich Chemie (Germany). Formic acid, acetonitrile, ethanol, water and all other solvents were of HPLC grade obtained from Merck.

Preparation of Standard solutions

Rutin and Quercetin: Standard stock solutions of the two flavonoids were prepared in ethanol at concentrations of 400µg/ml (10 mg of the standard was dissolved in ethanol, sonicated and volume was made up to 25 ml with the solvent to give 400µg/ml) and 2.5ml of the stock solution was made up to 10ml with ethanol to give a final concentration of 100µg/ml.

Caffeic acid and Chlorogenic acid: Standard solutions were prepared by dissolving 10mg of each of the standards in ethanol, sonicated and volume was made up to 25 ml with the solvent to give a concentration of 400µg/ml.

Plant material and preparation of the sample extract

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. The extract was prepared by mixing 100g of seed powder with 350ml of ethanol, the solution was stirred regularly for 15 days and filtered. The filtrate was subjected to evaporation using rotary vacuum evaporator and the residue (3.5g) was dissolved in10ml of ethanol. Sample cleanup was done to remove the impurities using a C_{18} Sep-Pak cartridge and 20 µl aliquots were analyzed by HPLC.

Instrumentation and chromatographic conditions

The chromatography unit is a Shimadzu LC-20AD with a quaternary pump system and auto injector or auto sampler (SIL-20ACHT) with a column (VARIAN Pursuit XPs- C_{18}) dimensions of 250 x 4.6mm, 5µm, S/N 436, protected by a guard column. The unit is coupled to a UV/VIS detector (SPD-20A). RP-HPLC with C_{18} column is the most popular technique for the analysis of polyphenols of 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) (Kim and Lee, 2002) at a flow rate 0.9ml/min; column (VARIAN Pursuit XPs-C_{18}) dimension 250 x 4.6mm) at 40^{o}C temperature. An automated sample injector was used for 20µl of sample injection. The chromatogram of the sample was compared with the chromatogram of the standard to identify the peaks of flavonoids. The concentrations of the polyphenolics isolated were calculated by comparing the peak area of the sample with that of the standard. All the determinations were carried out in duplicates. At the end of the analysis of each day, the columnwas washed with 26% acetonitrile in water for 1 hour and then flushed with 100% methanol.

Table 1. Structures of phenolic compounds (caffeic acid, chlorogenic acid, quercetin and rutin) present in *Coriandrum sativum* (www.google.com)

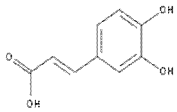
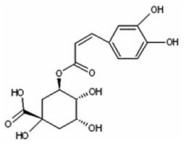
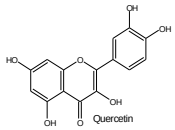
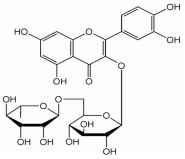
Sl No	Common name	Chemical name	Structural formula	Molecular weight	Structure
1	Caffeic acid	3,4-dihydroxy-cinnamic acid; trans-caffeate;3,4-dihydroxy-trans-cinnamate;(E)-3-(3,4-phenyl)-2-dihydroxypropenoic acid;3,4-dihydroxybenzeneacrylicacid; 3-(3,4-dihydroxyphenyl)-2-propenoic acid	$C_9H_8O_4$	180.16	
2	Chlorogenic acid	3R-[3-(3,4-dihydroxyphenyl)-1-oxo-2-propenyl]oxy]-1S,4R,5R-trihydroxy-cyclohexanecarboxylic acid	$C_{16}H_{18}O_9$	354.3	
3	Quercetin	2-(3,4- dihydroxyphenyl)- 3,5,7-trihydroxy- 4*H*- chromen- 4-on	$C_{15}H_{10}O_7$	302.236	
4	Rutin	3, 3', 4', 5, 7- pentahydroxy flavone-3-rutinoside, 3-rhamnosyl-glucosyl quercetin and 3-[[6-O-(6-deoxy-alpha-L-mannopyranosyl)-beta-D-glucopyranosyl] oxy]-2-(3, 4-dihydroxyphenyl)-5,7-dihydroxy-4*H*-1-benzopyran-4-one.	$C_{27}H_{30}O_{16}$	610.53	

Table 2. Retention time, peak area and concentration of the compounds

S. No	Compound	Retention time	Peak area of the standard	Peak area of the sample	Concentration (%)
1	Caffeic acid	4.048	35611226	912467	0.074
2	Chlorogenic acid	3.754	1688610	449472	0.45
3	Quercetin	11.637	9059782	223896	0.07
4	Rutin	2.602	1788621	856806	1.30

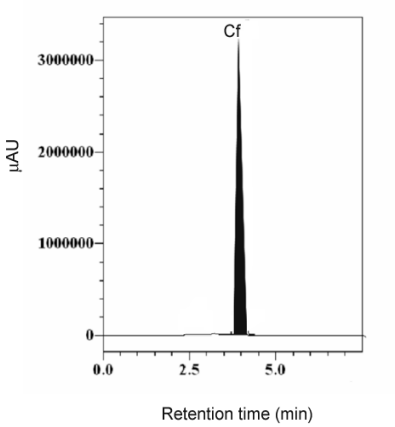


Fig.1 HPLC chromatogram of standardcaffeic acid

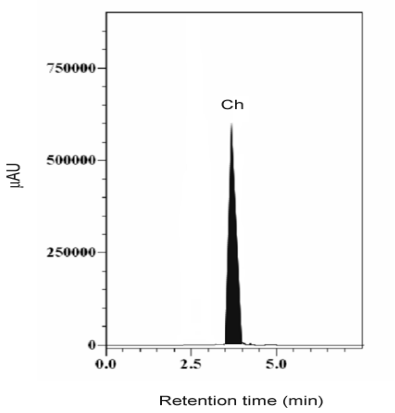


Fig.2 HPLC chromatogram of standard chlorogenic acid

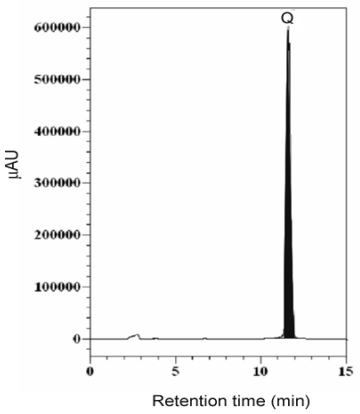


Fig.3 HPLC chromatogram of standard quercetin

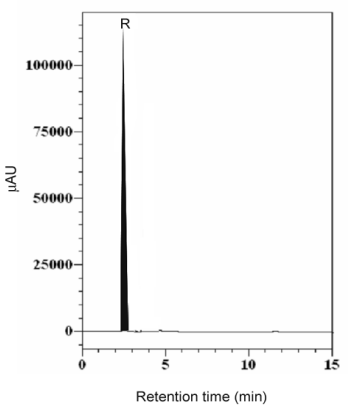


Fig.4 HPLC chromatogram of standard rutin

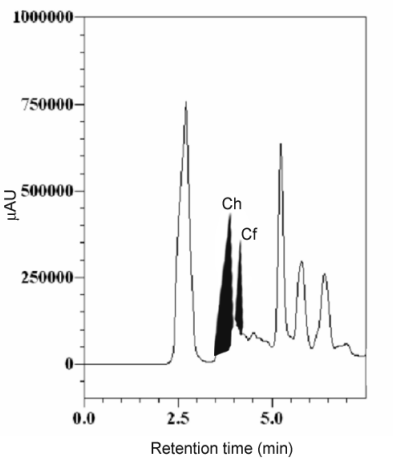


Fig.5 HPLC chromatogram of ethanolic extract (Detection at 320nm, peaks: Ch-chlorogenic acid, Cf-caffeic acid)

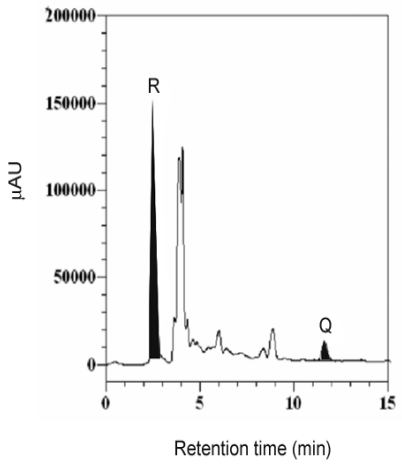


Fig.6 HPLC chromatogram of ethanolic extract (Detection at 370nm, peaks : R-rutin, Q-quercetin)

RESULTS

Figure 1, 2, 3 and 4 (above) represent HPLC chromatograms of the standard caffeic acid, chlorogenic, quercetin and rutin respectively. **Fig 5** represents HPLC chromatograms of the extract showing chlorogenic acid and caffeic acid and **Fig 6** represents that of rutin and quercetin in the ethanolic extract of coriander seeds. The retention time (RT) of the standards viz. chlorogenic acid, caffeic acid, quercetin and rutin, area under standard peaks as well as sample peaks and concentration of various compounds under investigation are presented in **Table 2**.

DISCUSSION

Over the past decade, evidence has accumulated that plant polyphenols and especially flavonoids are the most important class of defence antioxidants. With several endogenous antioxidants, they play a role in optimum protection from oxidative stress caused by an increase in the level of reactive oxygen species (ROS) in the human body. The results from several epidemiological studies provide support for a protective effect of dietary intake of flavonoids against diseases (Middleton & Kandaswami, 1993). This has led to more interest in searching for rich plant sources of flavonoids and for simple and accurate methods of analysis of flavonoids.

The variety of flavonoids occurring in plant materials is usually large; the components of flavonoid fractions come from different classes of aglycone, mono and polyglycoside, or acylated compound and differ from each other in polarity, molecular weight, and chromatographic and spectrophotometric properties. Every plant has an original and unique flavonoid profile, which makes quantification difficult. For this reason, the methods frequently used to determine the total flavonoid content of herbal materials include hydrolysis of the glycosides to reduce the variety and number of analytes. The aglycones obtained can then be quantified by UV spectrophotometric determination as Aluminium chelate complexes, as described in several pharmacopoeias (European Pharmacopoeia, 2004). For many herbal drugs, however, this method is reproducible and accurate, owing to provide good resolution and quantification of the flavonoid compounds.

In the course of optimization of the methods for separation and analysis of the flavonoid aglycones rutin, quercetin, chlorogenic acid and caffeic the in the ethanolic extract of the seeds of *Coriandrum sativum* L. through reversed-phase high performance liquid chromatography (RP-HPLC) with UV detection, good resolution of the flavonoids was achieved with different combinations of isocratic and gradient techniques. 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 ethanolic extract of *Coriandrum sativum* L. seeds.

Compounds in the ethanolic extract were identified by comparing the retention times with that of the standards and the extract contained mainly 4 polyphenolic compounds. **Fig 5** represents HPLC chromatograms of chlorogenic acid and caffeic acid and **Fig 6** represents that of rutin and quercetin in the ethanolic extract of coriander seeds. The amounts of polyphenols are in the order of rutin> chlorogenic acid >caffeic acid >quercetin in the prepared multi-component extract as shown in **Table 2.**

CONCLUSION

Ethanolic extract of coriander seeds is a good source of flavonoids containing rutin, chlorogenic acid, caffeic acid and quercetin, rutin being the highest and quercetin being the lowest in quantities. The method presented is simple, precise, selective and reproducible for the identification and determination of poyphenolics viz. caffeic acid, chlorogenic acid, quercetin and rutin in the ethanolic extract of coriander seeds. The additional peaks seen in the sample chromatograms indicate the presence of some soluble compounds extracted along with the polyphenolic compounds under study which warrants further investigation to identify the compounds in the ethanolic extract of coriander seeds.

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