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Research Article

Comparative analysis of antioxidant activities and phytochemical properties of some culinary herbs

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Abstract

The present work aimed to evaluate the antioxidant activities as well as phytochemical analysis of leaf extracts of some commonly used leafy spices such as *Murraya koenigii (Mk), Coriandrum sativum (Cs), Trigonella foenum-graecum (Tfg)* and *Mentha* x *piperita (Mp)*. Lyophilised plant extracts (LPEs) were obtained by hot water extraction (HWE) process followed by rotavap and lyophilisation. Among the herbs tested, *Mk* showed the highest antioxidant activity in DPPH scavenging (77.35 % mg^{-1} of LPE), superoxide anion radical scavenging (60.21 % mg^{-1} of LPE) and hydrogen peroxide scavenging (57.21 % mg^{-1} of LPE) model. *Tfg* showed least activity in DPPH scavenging (33.15 % mg^{-1} of LPE) and superoxide anion radical scavenging (25.36 % mg^{-1} of LPE) assay while *Cs* had the least activity in hydrogen peroxide scavenging (43.70 % mg^{-1} of LPE) system. Phytochemical investigations revealed the presence of major primary and secondary metabolites. *Mk* possessed highest amount of phenolics (5.70 mg GAE g^{-1} of LPE), soluble sugars (68.18 mg GLE g^{-1} of FTW) and proteins (69.84 mg BSAE g^{-1} of FTW) and plant pigments (total chlorophyll 6.22 mg g^{-1} of FTW and total carotenoid 0.19 µg g^{-1} of FTW) among the herbs. SDS-PAGE and HPLC finger printing had been performed for analysis of protein patterns and phenolic compounds respectively. In conclusion, addition of culinary herbs and leafy spices that show high to moderate antioxidant activity with an excellent amount of phytochemicals in dietary items would go a long way in assuring human health and wellness as well as enhancement of the disease fighting capacity against oxidative stress related disorders.

Key words: Culinary herbs, Leafy spices, Antioxidant activity, SDS-PAGE, HPLC, Phytochemicals, Human health, Oxidative stress related disorders.

Introduction

Currently, one of the most numerously pronounced keyword related to food-health-disease concept is ROS i.e., Reactive Oxygen Species. ROS chemically includes all those oxygenated free radical (OFR) species exemplified as singlet oxygen ($^1O_2$), superoxide anion ($O_2^{\bullet-}$), hydroxyl radicals ($OH^\bullet$), peroxyl radicals ($ROO^\bullet$), nitric oxide radical ($NO^\bullet$), peroxynitrite ($ONOO^\bullet$) as well as some non-radical forms (hydrogen peroxide, H_2O_2; hypochlorous acid, HOCl), frequently generated in biological systems by endogenous or exogenous factors. ROS, surprisingly besides playing super roles in energy production, phagocytosis, regulation of cell growth and intercellular signalling, or synthesis of biologically important compounds (Halliwell, 1997), hammer the living systems when produced in excess, resulting in oxidative stress and causing oxidation of cellular biomolecules *viz.* carbohydrates, proteins, lipids and nucleic acids; membrane damage, decreasing membrane fluidity. Such damage in turn leads to several ROS-mediated diseases or disorders such as cancer, acquired immunodeficiency syndrome, malaria, cardiovascular disease, stroke, gastric ulcer, diabetes, malignant tumours, rheumatic joint inflammation, arthritis, cataracts, Parkinson's and Alzheimer's disease, old-age symptoms and aging etc. (Halliwell and Gutteridge, 1984; Maxwell, 1995; Halliwell, 2000; Young and Woodside, 2001; Moskovitz *et al.*, 2002; Heinecke, 2003). Scientific reports advocate that these diseases or disorders can be controlled or cured by regular intake of dietary antioxidants (Atoui *et al.*, 2005; Alasalvar *et al.*, 2005) as antioxidant molecules scavenge free radicals by inhibiting initiation and breaking chain propagation or suppressing the formation of free radicals by binding to the metal ions, quenching hydrogen peroxide and

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superoxide and singlet oxygen (Shi *et al.*, 2001). Natural antioxidants present in dietary food-adjuncts or foodstuffs have only recently attracted attention because of their presumed safety concern as well as high therapeutic attributes. Different parts and products of plant such as fruits, vegetables, culinary herbs and spice etc. contain a wide variety of phytochemicals and provide a storehouse of natural antioxidants. Phytochemicals such as carotenoids, phenolic compounds, flavonoids, terpenoids, alkaloids, nitrogen compounds, vitamins, and some enzymes show remarkable antioxidant potentiality.

In most of the contemporary cuisine culinary herbs and leafy spices have been employed to impart diverse flavor, aroma, color and taste to various foods and drinks around the world. Foods and drinks in combination with culinary herbs are full of pharmacological agents; they act as drugs in the body and strengthen body's defense system. Ineffective defense system may lead to a high-risk of various disease developments. Majority of the present day diseases are reported to be due to the shift in the balance of the pro-oxidant and the antioxidant homeostatic conditions in the body. Pro-oxidant conditions dominate either due to the increased generation of the free radicals caused by excessive oxidative stress of sedentary lifestyle, or due to the poor scavenging system in the body caused by the depletion of the dietary antioxidants (Schulz *et al.*, 2000; Dringen, 2000). In diet-based therapies, research investigations have been carried out to evaluate the medicinal importance of various culinary herbs and leafy spices. Hence the present study attempts to assess the healing power of culinary herbs and leafy spices in terms of antioxidant potentiality and explore their use in daily lives for the benefits of human health and management of diseases.

Materials and methods

Plant materials and sample collection

Fresh leaves or aerial parts of curry tree, coriander, fenugreek and peppermint were collected from local vegetable super market, Shivmandir (26°42'29.63" N and 88°21'40.52" E) Siliguri, Darjeeling. Plant specimens were identified and authenticated taxonomically by Prof. A.P. Das, Department of Botany, University of North Bengal, Siliguri, WB and Herbarium specimens were prepared (Table 1).

Extraction and preparation of lyophilized extract

For HWE, a modified method after Aliakbarlu and Tajik (2012) was followed. A 10 g of freshly washed and finely chopped leaf samples was extracted for 30 min under darkness by refluxing with HPLC grade deoxygenated water (1:10, w/v) at 100 °C in a temperature controlled water bath shaker with gentle agitation. After cooling, each sample was filtered through Whatman filter paper (Grade 1) and the solid residues obtained were further treated with same procedure twice. The filtrate fractions from every extraction process were pooled and concentrated under reduced pressure at 40 °C in a rotary evaporator equipped with chiller, followed by lyophilisation in a vacuum freeze-dryer to obtain the lyophilized crude extracts. The lyophilised extracts were weighed and re-dissolved in same fluid to prepare stock solutions of desired concentrations and subsequently stored in air tight vials at -20°C until use for analyses.

Determination of water soluble extractive value

The water soluble extractive (WSE) value was expressed in percentage (%) and was determined using the formula: % WSE = ($Weight_{lyophilised\ crude\ extract}$ / $Weight_{initial\ plant\ material}$) X 100. Water-soluble extractive value plays an important role in evaluation of crude plant extracts. It was observed that highest WSE value was in *M. koenigii* (7.73 %), followed by *M. piperita* (5.75 %) and *T. foenum-graecum* (4.96 %), and lowest in *C. sativum* (2.05 %).

Determination of total moisture content

Moisture content of leaf samples was determined using a laboratory oven kept at 105 ± 3 °C for 24 h. The moisture content (%) was calculated according to AOAC (1975), using the following formula: Total moisture

Table 1. List of the herbs used in the present study along with their reported uses

English common name	Bengali vernacular name	Scientific name	Taxonomic family	Culinary use	Medicinal use
Curry tree	Kari pata	*Murraya koenigii* (L.) Spreng.	Rutaceae	As spice in different food preparations, curry powder, pickle, chutney, sausages and seasonings.	For curing dysentery, vomiting; essential oil from leaves exhibited a strong antibacterial and antifungal activity.
Coriander	Dhonay	*Coriandrum sativum* L.	Apiaceae	As seasoning in curries, salads and soup; a garnish on cooked dishes; in gravies and as green curry paste.	An appetite stimulant; leaves have antibacterial and antifungal properties; useful for headaches, muscle pain, stiffness and arthritis.
Fenugreek	Methi	*Trigonella foenum-graecum* L.	Fabaceae	In spicy soups and stews; as garnish; seasoning in curries, salads and sauses; microgreens used in salads.	Leaf showed anticholesterolemic, anti-inflammatory, antitumor, carminative, expectorant, febrifuge, hypoglycaemic, parasiticide.
Peppermint	Pudina	*Mentha* x *piperita* L.	Lamiaceae	In foods and drinks preparation, confectioneries and sweet liquors, sauces and salads; as garnish and stuffing.	Pain reliever, stimulating, stomachic, carminative, anti-spasmodic, treatment of cholera and diarrhoea.

content (%) = [(Weight$_{initial}$ - Weight$_{final}$)/ Weight$_{initial}$] x 100. The experiment was performed in triplicates (n = 3).

Analysis of antioxidant activities

Determination of total polyphenol content

The total polyphenol content was assayed spectrophotometrically at λ_{750} with FCR using gallic acid as the standard (Taga *et al.*, 1984). The total polyphenol content (TPC) was calculated as gallic acid equivalents (GAE) from a calibration curve of gallic acid standard solutions and expressed as mg of GAE g^{-1} of LPE. The experiment was performed in triplicates (n = 3).

Determination of total flavonoid content

Total flavonoid content was estimated spectrophotometrically at λ_{420} using the method described by Ordon ez *et al.* (2006). Total flavonoid content (TFC) was calculated as catechin equivalents (CAE) from a calibration curve of (+)-catechin standard solutions and expressed as mg of CAE g^{-1} of LPE. The experiment was performed in triplicates (n = 3).

DPPH' free radical scavenging activity

The DPPH' scavenging activity was monitored at λ_{517} using the method of Yen & Duh (1994), with slight changes. Free radical scavenging (FRS) activity expressed as percentage

inhibition (% I) of the DPPH• radical was calculated according to the formula given by Viuda-Martos *et al.* (2010): FRS activity (% I) = $[(A_c - A_s)/ A_c] \times 100$, where A_c refers to the absorbance of control (t = 0 min) and A_s is the absorbance of sample plus DPPH• (t = 30 min). The experiment was performed in triplicates ($n = 3$).

Superoxide anion scavenging activity

The superoxide anion radicals ($O_2^{\cdot-}$) scavenging activity was determined according to the method described by Nishikimi *et al.* (1972). Percentage of $O_2^{\cdot-}$ scavenged at λ_{560} was measured using the formula: Superoxide anion scavenging (SAS) activity (% I) = $[(A_0 - A_s)/A_0] \times 100$, where A_0 was the absorbance of control, and A_s was the absorbance of sample extract at λ_{560}. The experiment was performed in triplicates ($n = 3$).

Hydrogen peroxide scavenging activity

The hydrogen peroxide (H_2O_2) scavenging activity was carried out following the procedure of Ruch *et al.* (1989). The percentage of H_2O_2 scavenging at λ_{230} by the extracts and standard were calculated using the following equation: H_2O_2 scavenging (HPS) activity (%) = $[(A_c - A_s) / A_c] \times 100$, where A_c was the absorbance of control and A_s was the absorbance of test sample at λ_{230}. The experiment was performed in triplicates ($n = 3$).

Phytochemical screening of extracts

Qualitative analysis of phytochemicals

Phytochemical screening to detect the presence or absence of some significant phytochemicals *viz.* phenols, flavonoids, tannins, alkaloids, cardiac glycosides, saponins, terpenes, steroid, etc. were performed according to standard methods (Harborne, 1973; Trease and Evans, 1989; Sofowora, 1993). The tests were based on the visual observation of colour change, chomophor formation or formation of a precipitation after addition of specific reagents or treatments.

Quantitative analysis of phytochemicals

Estimation of total soluble sugar content

For the estimation of total soluble sugar content, Anthrone's method as described by Plummer (1978) was followed. Total soluble sugar (TSS) content was calculated from a D-glucose calibration curve and results were expressed as mg of glucose equivalents (GLE) g^{-1} of fresh tissue weight (FTW). The experiment was performed in triplicates ($n = 3$).

Estimation of total soluble protein content

Total soluble protein was extracted using the standard protocol given by Chakraborty *et al.* (1995) and quantification was done according to Lowry *et al.* (1951) using BSA as standard. Total soluble protein (TSP) content was calculated as BSA equivalents (BSAE) from a calibration curve of BSA and expressed as mg of BSAE g^{-1} of fresh tissue weight (FTW). The experiment was performed in triplicates ($n = 3$).

Estimation of total carotenoid content

Carotenoids were extracted and estimated according to the method given by Lichtenthaler (1987). Absorbances of the sample were observed spectrophotometrically at λ_{645}, λ_{663} and λ_{480} and the total carotenoid content (TCR) was calculated by using the formula: TCR = $A_{480} - (0.114\ A_{663} - 0.638\ A_{645})$ µg g^{-1} fresh tissue weight (FTW). The experiment was performed in triplicates ($n = 3$).

Estimation of total chlorophyll content

Chlorophyll was extracted according to the method of Harbone (1998). Total chlorophyll content was estimated by observing the absorbance at 645 nm and 663 nm and calculated by the formula: total chlorophyll content (TCL) = $(20.2\ A_{645} + 8.02\ A_{663})$ mg g^{-1} fresh tissue weight (FTW). The experiment was performed in triplicates ($n = 3$).

SDS-PAGE analysis for protein pattern

To analyze the protein patterns of the samples, SDS-PAGE was performed on 10% resolving gels, as described by Sambrook *et al.* (1989).

HPLC fingerprint analysis for Phenolic compounds

For HPLC fingerprint analysis of phenolic compounds present in leaf extracts, a Shimadzu system (Shimadzu Corp., Kyoto Japan) was used. A flow rate of 1 ml min^{-1}, and gradient elution of acetonitrile-water-acetic acid (10:86:4, v/v/v) (solvent A) and of acetonitrile-water-acetic acid (80:16:4, v/v/v) (solvent B). 0-50 min solvent B from 0-100%; and injection volume of 20 µl were applied; whereas the separation of compounds was monitored at 280 nm and 320 nm (Pari and Latha, 2004).

Table 2. Qualitative detection of phytochemicals

Phytochemical category	*M. koenigii*	*C. sativum*	*T. foenum-graecum*	*M. piperita*
Reducing sugars	+	+	+	+
Phenols	+	+	+	+
Flavonoids	+	+	+	+
Tannins	+	+	-	+
Alkaloides	+	+	+	-
Cardiac glycosides	+	+	+	+
Saponins	-	-	+	+
Terpenes	-	-	-	-
Steroids	+	+	+	-
Anthraquinones	+	-	+	-
Vitamin C	+	+	+	+

+ = present, - = absent

Statistical analysis of data

Experimental analyses were carried out in triplicate (n=3) and data ware expressed as mean ± standard deviation (SD). Statistical analysis was carried out by SPSS software (IBM SPSS, USA). One-way analysis of variance was performed by ANOVA procedures.

Result and Discussion

Because of their enchanting flavoral and toptastic qualities, culinary herbs and leafy spices have always been prized to Indian cuisine. Besides, these botanicals are an excellent source of versatile phytochemicals which have been reported to show good antioxidant activity. Natural antioxidants present in culinary herbs and leafy spices are responsible for inhibiting or preventing the deadly effects of oxidative stress. Herbs and spices contain free radical scavengers like polyphenols, phenolic acids and flavonoids. Polyphenols form a complex group of molecules associated with the cell walls of most plant species. This group of compounds ranges from simple phenolic acids (e.g., caffeic acid) to high-molecular-weight tannins. Polyphenols have various applications, such as in the production of paints, paper, cosmetics, as tanning agents, and as natural colorants and preservatives in the food industry. In addition, some phenolic compounds are antibiotics and anti-diarrheal, antiulcer, and anti-inflammatory agents and can be used in the treatment of diseases such as hypertension, vascular fragility, allergies, and hypercholesterolemia (Bravo, 1998; Higdon and Frei, 2003).

Preliminary phytochemical study of the extracts from *M. koenigii, C. sativum, T. foenum-graecum* and *M. piperita* showed the presence of major plant secondary metabolites *viz.* reducing sugars, phenolic compounds, flavonoids, cardiac glycosides and vitamin C, etc. but tannins, alkaloids, saponins, steroids and anthraquinones occurred in some of them (Table 2). Such secondary metabolites contribute significantly towards the biological activities of plant extracts such as antioxidant, antimicrobial, hypoglycemic, antidiabetic, anti-inflammatory activities etc. (Negi *et al.*, 2011).

In vitro antioxidant activity, principally, can be determined by hydrogen atom transfer (HAT) method and single electron transfer (SET) or electron transfer (ET) method (Joon & Shibamoto, 2009). HAT based methods measure the ability of an antioxidant to scavenge free radical by hydrogen donation to form a stable compound. SET based methods detect the ability of the antioxidant to transfer one electron to reduce compound including metals, carbonyls and radicals (Prior *et al.*, 2005; Huang *et al.*, 2005). Superoxide anion scavenging (SAS) assay, hydrogen peroxide

scavenging (HPS) assay, etc. involve HAT method, and the assays of total polyphenolic content (TPC), total flavonoid content (TFC) etc. are of ET method, while DPPH• assay include both the method predominantly via SET method (Karadag *et al.*, 2009; Badarinath *et al.*, 2010). The relatively stable radical DPPH has been used widely for the determination of primary antioxidant activity, that is, the free radical scavenging activities of pure antioxidant compounds, medicinal plants and fruit extracts and food materials (Purohit *et al.*, 2005). Superoxide anion is a weak oxidant that gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress. Superoxide anion scavenging activity is correlated to the total flavonoids (Thaipong *et al.*, 2006). Hydrogen Peroxide radical scavenging activity is correlated to the presence of phenolic compounds. Generally, extracts that contain a high amount of phenolic compounds exhibit high antioxidant activity.

Antioxidant activity in terms of scavenging potentiality of hot water extracts of *M. koenigii*, *C. sativum*, *T. foenum-graecum* and *M. piperita* have been evaluated. All of them could act as potential radical scavengers in a concentration oriented fashion (Fig. 1). Interestingly *M. koenigii* showed the highest antioxidant activities scoring 77.354 % mg^{-1} of LPE in DPPH scavenging, 60.205 % mg^{-1} of LPE in superoxide anion radical scavenging and 57.209 % mg^{-1} of LPE in hydrogen peroxide scavenging assay, followed by *M. piperita*. Extract from *T. foenum-graecum* showed least scavenging activity in DPPH scavenging (33.145 % mg^{-1} of LPE) and superoxide anion radical scavenging (25.364 % mg^{-1} of LPE) system while *C. sativum* had the least activity in hydrogen peroxide scavenging assay (43.695 % mg^{-1} of LPE) .

The quantitative estimation of total moisture content, total soluble sugar, total soluble protein contents, total chlorophyll content, total carotenoid content present in the herb samples have been depicted in Table 3. Total moisture content among the herb samples was found in a range of 89% to 70%.The total soluble sugar content of was found highest in *M. koenigii* (68.18 mg GLE g^{-1} of FTW) and lowest in *T. foenum-graecum* (53.17 mg GLE g^{-1} of FTW), while content of

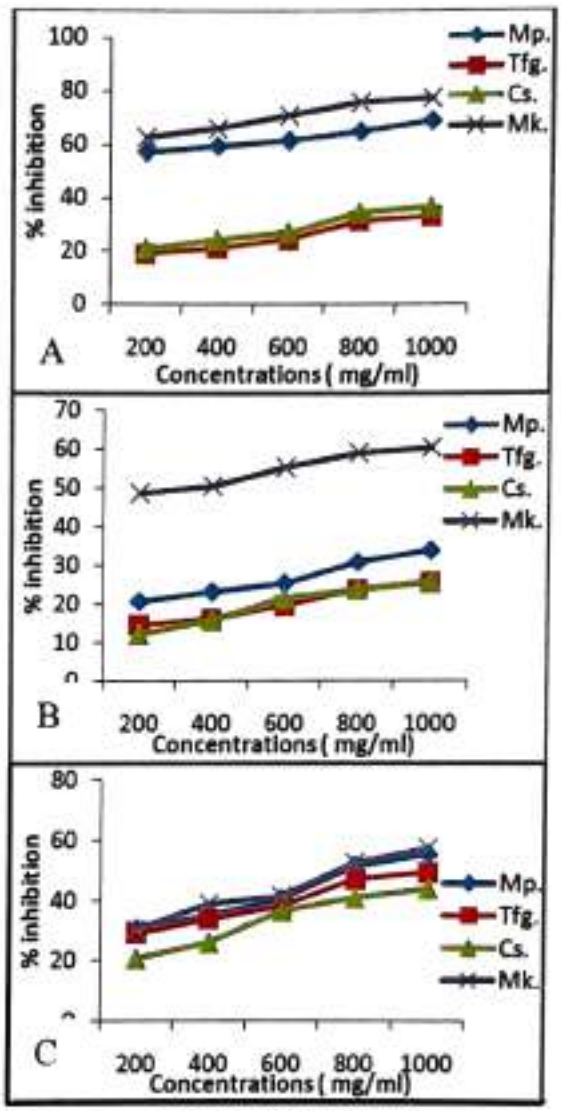

Fig. 1. Scavenging activity of the herb extracts. A. DPPH scavenging activity, B. Superoxide anion scavenging activity, and C. Hydrogen peroxide scavenging activity.

soluble protein was highest in *M. koenigii* (69.507 mg BSAE g^{-1} of FTW) and lowest in *C. sativum* (35.027 mg BSAE g^{-1} of FTW). Pigment analysis revealed that *M. koenigii* contained highest amount of total chlorophyll (6.223 mg g^{-1} of FTW) and carotenoid (0.190 µg g^{-1} of FTW) content. *T. foenum-graecum* and *M. piperita* showed the lowest chlorophyll content (1.639 mg g^{-1} of FTW) and lowest carotenoid content (0.063 µg g^{-1} of FTW) respectively. Garg *et al.*, 2012 also reported that chlorophyll content was higher in curry

leaves due to the darker shade of green, then the coriander.

Chlorophyll has been suggested as an effective antioxidant since it scavenges free radicals such as 1, 1-diphenyl-2-picrylhydrazyl (Khalaf *et al.*, 2008). Carotenoids that include xanthophylls and carotenes have the ability to detoxify various forms of activated oxygen and triplet chlorophyll that are produced as a result of excitation of the photosynthetic complexes by light. Dietary carotenoids are thought to provide health benefits due to their role as antioxidant molecules. SDS-PAGE analysis of proteins from the four herbs revealed the presence of a large number of bands in all cases, but not much difference was obtained among the herbs (Fig. 2).

The study also revealed that among the herbs *M. koenigii* ranked highest and *C. sativum* was lowest in total phenolic content and total flavonoid content (Table 4).Total phenolic content and total flavonoid content in extracts of *M. koenigii* was found as 5.70 mg GAE g^{-1} of LPE and 1.68 mg CAE g^{-1} of LPE. Leaf extract from *C. sativum* contained 2.55 mg GAE g^{-1} of LPE as total phenolic content and 0.66 mg CAE g^{-1} of LPE as total flavonoid content. HPLC analysis of the phenols from all four herbs was carried out and results are shown in fig. 3. Among the four, maximum number of peaks was obtained in *M.koenigii* and the least in *M.piperita*. The health benefit of phenolics is directly linked to their antioxidant potentiality. Phenolics act as effective antioxidants is mainly due to their redox properties, which allow them to behave as reducing agents, hydrogen donors, and singlet oxygen quenchers. The potential hazard from oxidative stress in the body may be compensated through the consumption of a diet exclusively rich in antioxidant phenolics including polyphenols, phenolic acids and

Table 3. Nutritional and pigment contents of the herbs

List of herbs	Total moisture content[a]	Total soluble sugar content[b]	Total soluble protein content[c]	Total chlorophyll content[d]	Total carotenoid content[e]
M. koenigii	78.83 ± 0.16	68.18 ± 0.87	69.84 ± 0.39	6.22 ± 0.37	0.19 ± 0.01
C. sativum	82.11 ± 0.67	62.55 ± 0.65	35.00 ± 0.45	2.336 ± 0.10	0.12 ± 0.00
T. foenum-graecum	70.41 ± 0.72	54.17 ± 0.57	35.03 ± 0.53	1.639 ± 0.09	0.07 ± 0.00
M. piperita	89.56 ± 0.50	56.42 ± 0.07	45.53 ± 0.61	2.040 ± 0.02	0.06 ± 0.00

Content expressed as mean ± SD in a. percentage, b. mg GLE g^{-1} of FTW, c. mg BSAE g^{-1} of FTW, d. mg g^{-1} of FTW, e. µg g^{-1} of FTW. Fresh tissue weight is abbreviated as FTW.

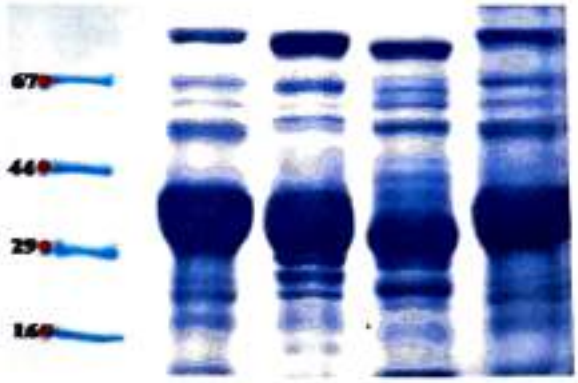

Fig. 2. SDS-PAGE analysis of protein of the herbs. Lane 1- Molecular weight markers in KDa; 2 - Tfg; 3- Mp; 4- Cs; 5-Mk

Table 4. Total phenol and flavonoid contents[#].

Herb samples	TPC	TFC
M. koenigii	5.70 ± 0.20	1.68 ± 0.26
C. sativum	2.55 ± 0.05	0.66 ± 0.12
T. foenum-graecum	3.45 ± 0.10	1.33 ± 0.18
M. piperita	5.06 ± 0.01	1.75 ± 0.18

[#]Content expressed as mean ± SD, TPC as mg GAE g^{-1} of LPE and TFC as mg CAE g^{-1} of LPE.

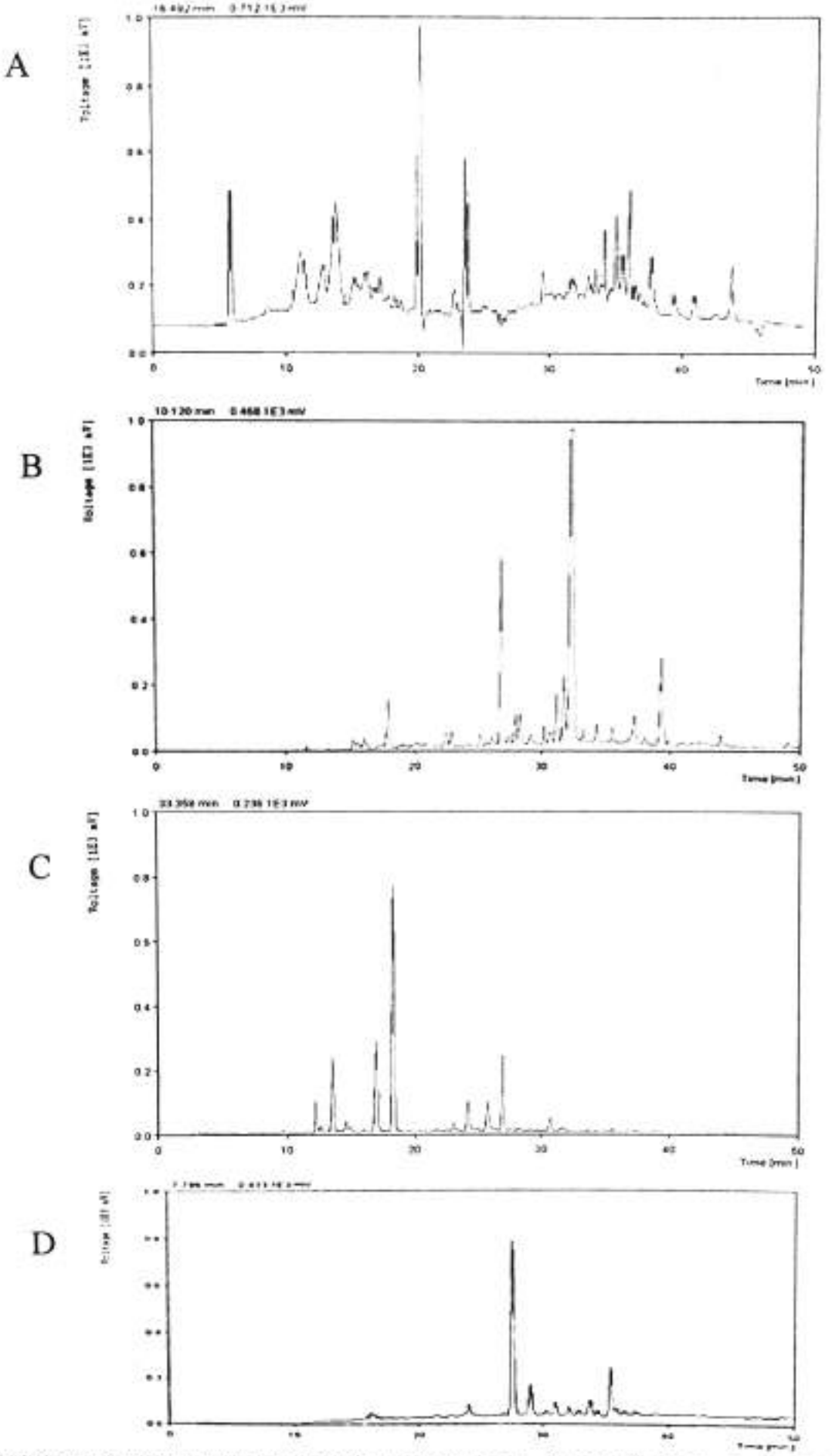

Fig. 3. HPLC chromatogram of phenolic compounds of herb extracts. A. *M. koenigii* B. *C. sativum* , C. *T. foenum-graecum* and D. *M. piperita.*

flavonoids. According to Scalbert and Williamson (2000) the amount of total human intake of phenolic compounds is about 1 g day^{-1} consisting two-thirds of flavonoids and one-thirds of phenolic acids.

Conclusion

Recently much attention has been focused on the analysis of dietary foods and food components. Food beyond its basic nutritional values has played some functional effects on prevention of diseases and maintenance of good health. Functional foods as the concept "any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains" (Thomas and Earl, 1994) are the centre of attraction for searching newer well-accepted functional foods from culinary herbs and leafy spices. As culinary herbs and leafy spices are full of variety in bioactive phytochemicals including antioxidant molecules and nutricomponents they can be incorporated as functional foods in our everyday's diets to rejuvenate ourselves.

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