ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Phenolic Compounds and Antioxidant Capacity in Bulgarian Plans (dry seeds)

Valentina Lubomirova Christova-Bagdassarian[1], Kristine Samvel Bagdassarian[2] and Maria Stefanova Atanassova[3]

1. National Centre of Public Health and Analyses, Department "Public Health and Health risk", 15 Akad. Ivan Ev. Geshov blvd, 1431 Sofia, Bulgaria.
2. University of Birmingham, Edgbaston, Birmingham, B15 2TT United Kingdom.
3. Metallotechnica Ltd., 63 Shipchenski prohod Bul., 1574 Sofia, Bulgaria.

Manuscript Info

Manuscript History:

Received: 11 October 2013
Final Accepted: 24 October 2013
Published Online: November 2013

Key words:
Anethum graveolens (Dill), *Carum carv*i (Caraway) and *Coriandrum sativum* (Coriander), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), *Foeniculum vulgare* (Fennel), *Pimpinella anisum* (Anise), Rutin, Tannins, Total flavonoids, Total phenolics.

Corresponding Author

Valentina L. Christova-Bagdassarian

Abstract

Objective: Comparative evaluations of the phenolic compounds and antioxidant capacity to various seeds from the Apiaceae family to which belong: Foeniculum vulgare (Fennel), Anethum graveolens (Dill), Pimpinella anisum (Anise), Carum carvi (Caraway) and Coriandrum sativum (Coriander) were carried out.

Methods: The seeds were analysed for their tannins content by titrimetric method; rutin was determined spectrophotometrically by using ammonium molybdate; the total phenolics content was determined by using Folin-Ciocalteu assay; the total flavonoids were measured spectrophotometrically using the colorimetric reaction with aluminum (III) chloride. Antioxidant capacity was also analysed spectrophotometrically by a 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging effect of the seeds.

Results: The total phenolic and flavonoid contents of the *Foeniculum vulgare* (Fennel) varied between 115.96 mg GAE/100g and 68.10 mg CE/100g. This content for the *Coriandrum sativum* (Coriander) is lower (between 17.04 mg GAE/100g and 11.10 mg CE/100g, respecively). The content of rutin and tannins of the *Foeniculum vulgare* (Fennel) varied between 2.81 % and 1.52 %. It was found to be much higher than the rutin content of the *Coriandrum sativu* (Coriander) (between 0.99 % and 0.42 %, respectively). The highest radical scavenging effect was observed in the *Foeniculum vulgare* (Fennel) with IC50 of 113.19 mL/L.

Conclusions: In the present study, the seeds from the *Apiacea family* to which belong: *Foeniculum vulgare* (Fennel), *Anethum graveolens* (Dill), *Pimpinella anisum* (Anise), *Carum carv*i (Caraway) and *Coriandrum sativum* (Coriander), were studied as sources of natural antioxidants. The results from the antioxidant assays show that all seeds can act as radical scavengers to a certain extent. Further studies in this area are in progress.

Introduction

From ancient times, the herbs and aromatic spices have been used due to their culinary qualities and medicinal properties, including their antioxidant activity (Albano S.M. and Migel M.G., 2011). Seeds of many plants and herbs are evaluated to measure their levels of phenolic phytochemicals, containing high antioxidant activity (Kwon Y.I. et al., 2011). Phenolic phytochemicals are used by plants to protect them from abiotic and biotic stresses, but are equally beneficial to preventing and combating human chronic diseases linked to oxidative stress (Shetty K. and Wahlqvist M.L., 2005).

The Apiaceae or Umbelliferae is a family of usually aromatic plants with hollow stems commonly known as umbellifers (Sahebkar A. and Iranshahu M., 2010). The genus *Ferula*, belonging to the family Apiaceae, comprises about 170 species. These are produced from central Asia westward to northern Africa (Pimenov M.G. and Leonov M.V., 1993; Sahebkar A. and Iranshahu M., 2010). The Iranian flora comprises of 30 species of *Ferula*, of which some are endemic (Mozaffarian V., 1983, 1996; Sahebkar A. and Iranshahu M., 2010). The popular Persian name of the most species is "Koma" (Mozaffarian V., 1996; Sahebkar A. and Iranshahu M., 2010). Notable members of this family include *Anethum graveolens* (Dill), *Anthriscus cerefolium* (chervil), *Angelica* spp. (Angelica), *Apium gravolence* (celery), *Carum carvi* (caraway), *Coriandrum sativum* (coriander), *Cuminum cyminum* (cumin), *Foeniculum vulgare* (fennel), *Ferula gummosa* (galbanum), and *Pimpinella anisum* (anise). The aromatic smell of most species is due to the presence of essential oil or oleoresin in their different organs [Singh V. and Jain D.K., 2007; Sahebkar A. and Iranshahu M., 2010).

Plants from the family *Apiaceae* are commonly used as food, flavoring of foods and for medical purposes. In particular, the seeds from family *Apiaceae* are known to be used as a household remedy for complications such as hypertension (Gilani A.H. et al., 2005). Various beneficial effects have been observed for seed extracts belonging to the Apiaceae family and lot of them have been evaluated. Essential seed oils from the *Foeniculum vulgare* (Fennel), such as anethole and limonene, are used for medical purposes, and the seeds are also used as tranquilizers and tonics (Oktay M. et al., 2003). Aqueous extracts of fennel seeds are observed for their hypotensive effects in a dose related manner (Oktay M. et al., 2003). *Anethum graveolens* (Dill) seed extract is used to treat diarrhea, ingestion and common colds (Husain S.Z. et al, 2008), and Dill is also fed to cows and goats for improving milk production (Lans C. et al., 2007). *Coriandrum sativum* (Coriander) seeds are often used as food flavoring agent and to treat ulcers (Husain S.Z. et al, 2008). *Carum carvi* (Caraway) plant is beneficial in treatment and management of type II diabetes and cardiovascular diseases, and evokes beneficial effects on the elevation of lipids in the bloodstream (Lemhadri A. et al., 2006). The *Pimpinella anisum* (Anise) belongs to the Middle Eastern region, where it is used as an aromatic spice and to help in digestion (Arslan N. et al., 1982).

The chemistry of genus *Ferula* has been studied by many investigators. To date, more than 70 species of *Ferula* have been investigated chemically (Murray R.D.H. et al., 1982; Tamemoto K. et al., 2001; Kogure K. et al., 2004; Iranshahi M, et al., 2008; 2010; and Iranshahi M. et al., 2004, 2007, 2008, 2009; Sahebkar A. and Iranshahi M., 2010). The plants of this genus are well documented as a good source of biologically active compounds such as derivatives (Motai T. et al., 2004; Sahebkar A. and Iranshahu M., 2010), and sulfur containing compounds (Zhi-da M. et al., 1987; Rajanikanth B. et al., 1984; Al-Said M.S. et al., 1996; Iranshahi M. et al., 2003, 2006, 2009; Sahebkar A. and Iranshahi M., 2010; Sahebkar A. et al.).

The high content of polyphenols and vitamins that is due to high total phenolic and total antioxidant activity, gives an excellent rationale for using plant sources for medical purposes. It has been suggested that the high antioxidant activity potential is often due to certain phenolic compounds (Kiselova Y. et al., 2006). The usefulness of antioxidants in our diet could be described as the slowing down of the oxidation of fats (Yen G.C. and Duh P.D., 1994; Zheng W. and Wang SY., 2001), as well as being related to the free radicals or the active oxygen scavengers (Oktay M. et al., 2003). Natural sources of antioxidants have the capability to protect against free radicals and chronic diseases (Oktay M. et al., 2003), whereas synthetic sources of antioxidants are restricted due to their carcinogenicity (Zheng W. and Wang S.Y, 2001; Sallem F., 2010).

In this present study, the seed extracts of some Bulgarian plants: *Foeniculum vulgare* (Fennel), *Anethum graveolens* (Dill), *Pimpinella anisum* (Anise), *Carum carvi* (Caraway) and *Coriandrum sativum* (Coriander) have been evaluated for their total phenolics and flavonoids, rutin, tannins and the antioxidant capacity (DPPH) of the dry seeds.

MATERIALS AND METHODS

Plant material

The study covered some varieties of species from different regions of Bulgaria: *Foeniculum vulgare* (Fennel), *Anethum graveolens* (Dill), *Pimpinella anisum* (Anise), *Carum carv*i (Caraway) and *Coriandrum sativum* (Coriander). The sampling lasted one year according to the seasonality of harvesting for individual species. All sample data are stated in the sampling protocol. The dried seeds were kept in a dry place until further use.

Chemical reagents: Methanol HPLC grade; Gallic acid; (+)-Catechin; Folin-Ciocalteu's phenol reagent; Sodium carbonate, Sodium nitrite; Aluminium (III) chloride; Sodium hydroxide; Rutin; Ammonium molybdat; Indigo

carmine; 0.1 N Potassium permanganate (water solution); 96% - 98% Sulfuric acid; 2,2-diphenyl-1-picrylhydrazyl (DPPH·); Ascorbic acid. All chemicals were of analytical grade (Sigma Chem. Co.).

Sample preparation

The phenolic and flavonoid compounds were extracted from a 0.5 g dried sample by 50 mL 80% aqueous methanol in an ultrasonic bath for 20 minites. An aliquot (2 mL) of the extracts was ultracentrifugated for 5 minutes at 14 000 rpm (Marinova D. et al., 2005).

Determination of total phenolics assay

The total phenolic contents of dry herbs were determined by using the Folin-Ciocalteau assay. An aliquot (1 mL) of extract or standard solution of gallic acid (20, 40, 60, 80 and 100 mg/L) was added to 25 mL volumetric flask, containing 9 mL of destilled deionised water (dd water). A reagent blank (using dd water) was prepared. One milliliter of Folin-Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 minutes, 10 mL 7% Sodium carbonate solution was added to the mixture. The solution was diluted to the mark (25 mL) with dd water and shaken. After incubation for 90 minutes at room temperature, the absorbance against the reagent blank was determined at 750 nm using an UV-VIS Spectrophotometer CARY Varian (Varian Australian Pry. Ltd) . The total phenolic contents of the dry herbs were expressed as milligrams of gallic acid equivalents (GAE) per 100 grammes dry weight (mg GAE/100g dw). All samples were analysed in triplicate (Marinova D. et al., 2005).

Determination of total flavonoids assay

Total flavonoid contents were measured by aluminum chloride colorimetric assay. An aliquot (1 mL) of extracts or standard solution of (+)-catechin (20, 40, 60, 80 and 100 mg/L) was added to 10 mL volumetric flask, containing 4 mL dd water. To each flask was added 0.3 mL 5% sodium nitrite. After 5 min, 0.3 mL 10% aluminium (III) chloride was added. After six minutes, 2 mL 1 M Potassium permanganate was added and the total volume was made up to 10 ml with dd water. The solution was mixed well and the absorbance was measured against the reagent blank at 510 nm using an UV-VIS Spectrophotometer CARY Varian (Varian Australian Pry. Ltd). The results for the total flavonoid contents of dry herbs were expressed in milligrams of (+)-catechin equivalents (CE) per 100 grammes dry weight (mg CE/100g dw). All samples were analyzed in triplicate (Marinova D. et al., 2005).

Sample preparation

A sample (1 to 5 g) was extracted by 15 mL 80% methanol at room temperature. After filtration the samples were transfered into 50 mL volumetric flask and diluted to the volume by 80% methanol (Atanassova M. and Christova-Bagdassarian V., 2009).

Rutin assay

The rutin content in dry seeds were analyzed according to The International Pharmacopoeia and AOAC International method, modified using 80% aqueous methanol. The aliquot of 2 mL solution was pipetted into 50 mL volumetric flask, diluted to 2 mL with dd water and 5 mL ammonium molybdat was added. The solution was refilled to the mark (volume of 50 mL) with dd water and mixed. The rutin standard solution was prepared by dissolving 0.0200 g into 2 mL methanol and diluting to the volume of 50 mL with 80% aqueous methanol. An aliquot (1 mL) of standard solution was transferred into 50 mL volumetric flask and was diluted to the mark by dd water. A reagent blank (using dd water) was prepared. The absorbance against the reagent blank was measured at 360 nm by using an UV-Vis Spectrophotometer CARY Varian (Varian Australian Pry. Ltd). All samples were analyzed in triplicate (Atanassova M. and Christova-Bagdassarian V., 2009).

Calculations (based on the mean value, n=3):
The content (%) of rutin (R) in sample is done by formula:

$$R(\%) = \frac{A_{sample} \times C \times 50 \times 100}{A_{std} \times W \times 2}$$, where

A_{sample} –is the absorbance of the sample at 360 nm (average value, n=3), **A_{std}** is the absorbance of the standard solution at 360 nm, **C** is the concentration of the standard solution of rutin (g/mL), **W** is the weight (g) of the sample analysed, **2** – is the volume (mL) of the sample analysed, **100** - percents, %.

Sample preparation

The sample of dry herbs (3 g) was weighted, extracted with dd water into 250 mL volumetric flask at room temperature for 4 hours and filtered (Atanassova M. and Christova-Bagdassarian V., 2009).

Tannins assay

The analyses of tannins content in dry herbs were performed according to The International Pharmacopoeia and AOAC method, after modifiing. The volume of 25 mL of the sample extract was measured into 1 L conical flask, and then 25 mL indigo solution and 750 mL dd water were added. After mixing, the blue solution was titred with 0.1 N Potassium permanganate until a green color, and then a few drops were added at this time until the solution became golden yellow coloured. The standard solution of Indigo carmine was prepared (dissolve 6 g indigo carmine in 500 mL dd water by heating, cooling and add 50 mL 96% - 98% Sulfuric acid, dilute to 1 L and filter). Similarly, the mixture of 25 mL indigo carmine solution and 750 mL dd water was titred for the blank sample. All samples were analyzed in triplicate (Atanassova M. and Christova-Bagdassarian V., 2009).

Calculations (based on the mean value, n=3):
The content of tannins (T, %) in sample is done by formula:

$$T,\% = \frac{(V - V_0) \times 0.004157 \times 100}{g \times 25}$$, where

V is the volume of 0.1 N Potassium permanganate from titration of the sample, mL; **V_0** is the volume from 0.1 N Potassium permanganate for titration of blank sample, mL; **0.004157** is the Tannins equivalent coefficient for 1 mL of 0.1 N Potassium permanganate; **g** is the mass of the analysed sample, g; **250** is the volume of the volumetric flask, mL.

DPPH assay

The most commonly used antioxidant methods are ABTS· and DPPH. Both of them are characterized by excellent reproducibility under certain assay conditions, but they also show significant differences in their response to antioxidants. The DPPH free radical (DPPH·) does not require any special preparation, while the ABTS radical cation (ABTS·) has to be generated by enzymes or chemical reactions (Wojdylo A. et al., 2007). In the DPPH free radical method, the antioxidant efficiency is measured at the ambient temperature and thus eliminates the risk of thermal degradation of the molecules tested (Bondel V. et al, 1997). The hydrogen atom or electron donation abilities of the corresponding extracts and some pure compounds were measured from the bleaching of the purple-colored methanol solution of 1,1-diphenly-2-picrylhydrazyl (DPPH). This spectrophotometric assay uses the stable radical DPPH as a reagent. One thousand microlitres of various concentrations of the extracts in ethanol were added to 4 mL of 0.004% methanol solution of DPPH. After a 60 min incubation period at room temperature, the absorbance was measuread against a blank at 517 nm. All spectrophotometric data were acquired using a Helios β UV-Vis spectrophotometer (Unicam Specrophotometer, Great Britain). Disposable cuvettes (1 cm× l cm x 4.5cm) from Ratiolab (Dreieich, Germany) were used for visible absorbance measurements.

Calculations (based on the mean value, n=3):
Inhibition of free radical by DPPH in percent (IC %) was calculated by formula :

$IC,\% = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$, where

A_{blank} is the absorbance of the control reaction (containing all reagents, except the test compound); **A_{sample}** is the absorbance of the test compound.

Extract concentration providing 50% inhibition (IC50%) was calculated from the graph plotting the relation between the inhibition percentage and the extract concentration [Gezer K. et al., 2006; Bektas T.L. et al., 2007). The objectives of this study were to evaluate and compare total antioxidant capacity to some Bulgarian plants (dry seeds) the *Foeniculum vulgare* (Fennel), *Anethum graveolens* (Dill), *Pimpinella anisum* (Anise), *Carum carv*i (Caraway) and *Coriandrum sativum* (Coriander).

Statistical Analysis

All experiments were performed in triplicates. Analysis at every time point from each experiment was carried out in duplicate or triplicate. The statistical parameters are calculated in terms of the reproducibility of the experimental data using a statistical package universal ANOVA.

RESULTS

Different phytochemicals have various protective and therapeutic effects which are essential to prevent diseases and maintain a state of well being. The methanolic extract of *Foeniculum vulgare* (Fennel), *Anethum graveolens* (Dill), *Pimpinella anisum* (Anise), *Carum carvi* (Caraway) and *Coriandrum sativum* (Coriander) were analyzed for its phyroconstituents. The quantitative estimation of the phytochemical constituents of *Foeniculum vulgare* (Fennel), *Anethum graveolens* (Dill), *Pimpinella anisum* (Anise), *Carum carvi* (Caraway) and *Coriandrum sativum* (Coriander) show that the dry seeds are rich in total phenols, total flavonoids, rutin and tannins to some extent (data are shown in the Table 1 and Table 2). The presence of these phytochemicals in dry seeds is significant according to present study. The content for total phenolics and total flavonoids of *Foeniculum vulgare* (Fennel) varied between 115.96 mg GAE/100g and 68.10 mg CE/100g. It was found to be much higher than it was in *Coriandrum sativum* (Coriander), where the content ranged between 17.04 mg GAE/100g and 11.10 mg CE/100g, respectively. This is shown in the Table 1 using the gallic acid and catechin as standards.

These results indicate that the higher antioxidant activity of the methanol extract of the *Foeniculum vulgare* (Fennel) than the methanol extract of *Coriandrum sativum* (Coriander) may be in correlation with the phenolic and flavonoid contents of the extracts of .the same plant.

Table 1. Total phenolics and total flavonoids in the studied Bulgarian dry seeds.

Bulgarian dry seeds	**Total phenolics, (mg GAE /100g dw)**	**Total flavonoids, (mg CE /100g dw)**
Foeniculum vulgare (Fennel)	115.96±0.04 *(RSD 5.7; n=3)*	68.10±0.03 *(RSD 5.8; n=3)*
Anethum graveolens (Dill)	69.87±0.03 *(RSD 5.7; n=3)*	49.10±0.03 (RSD 7.5; n=3)
Pimpinella anisum (Anise)	46.17±0.03 *(RSD 7.7; n=3)*	17.43±0.03 *(RSD 8.6; n=3)*
*Carum carv*i (Caraway)	25.96±0.03 *(RSD 8.1; n=3)*	11.77±0.02 *(RSD 8.9; n=3)*
Coriandrum sativu (Coriander)	17.04±0.02 *(RSD 8.6; n=3)*	11.10±0.02 *(RSD 8.8; n=3)*

The presence of rutin and tannins in dry seeds is significant. The content for rutin and tannins in *Foeniculum vulgare* (Fennel) varied between 2.81 % and 1.52 % and it was found that it was much higher than the content in *Coriandrum sativu* (Coriander) (between 0.99 % and 0.42 %, respectively). The results were shown in the Table 2, where the data was received using rutin as standard and Potassium permanganate as titrant. It is important to notice that the comparison of the results for rutin and tannin contents in the dry seeds will not be correct because of the different methods of analysis.

Table 2. Rutin and tannins in the studied Bulgarian dry seeds

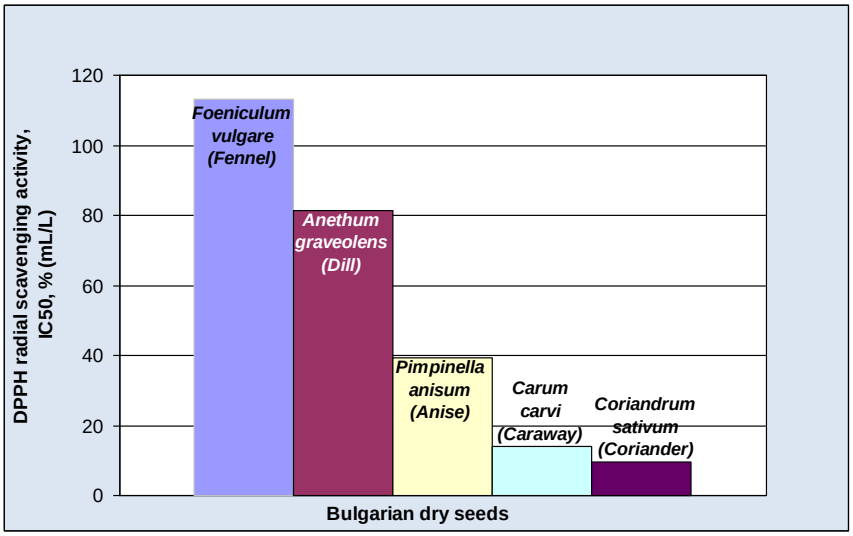
Bulgarian dry seeds	Rutin, %	Tannins, %
Foeniculum vulgare (Fennel)	2.81±0.004 *(RSD 7.8; n=3)*	1.52±0.003 *(RSD 7.6; n=3)*
Anethum graveolens (Dill)	1.77±0.003 *(RSD 7.9; n=3)*	1.11±0.003 *(RSD 7.9; n=3)*
Pimpinella anisum (Anise)	1.18±0.002 *(RSD 7.4; n=3)*	0.83±0.002 *(RSD 8.6; n=3)*
*Carum carv*i (Caraway)	1.02±0.002 *(RSD 8.7; n=3)*	0.55±0.002 *(RSD 8.7; n=3)*
Coriandrum sativu (Coriander)	0.99±0.002 *(RSD 8.8; n=3)*	0.42±0.002 *(RSD 9.1; n=3)*

The methanolic extract was subjected to screening for their possible antioxidant activity. The $DPPH^+$ assay provided information on the reactivity of test compounds with a stable free radical. Because of its odd electron $DPPH^+$ gives a strong absorption band at 517 nm in visible spectroscopy (deep violet color). As this electron becomes paired off in the presence of a free radical scavenger, the absorption vanishes, and the resulting decolorization is stoichiometric with respect to the number of electrons taken up. To evaluate the scavenging effect of $DPPH^+$ on methanolic extract of *Foeniculum vulgare* (Fennel), *Anethum graveolens* (Dill), *Pimpinella anisum* (Anise), *Carum carv*i (Caraway) and *Coriandrum sativum* (Coriander), $DPPH^+$ inhibition was investigated and these results are shown in Table 3 and Figure 1 as relative activities against control.

Table 3. DPPH radical scavenging activity of Bulgarian dry seeds

Bulgarian dry seeds	DPPH radical scavenging activity, IC50% (mL/L)
Foeniculum vulgare (Fennel)	113.19±0.03 *RSD* 4.4% *(n=3)*
Anethum graveolens (Dill)	81.52±0.02 *RSD* 5.2% *(n=3)*
Pimpinella anisum (Anise)	39.36±0.03 *RSD* 4.6% *(n=3)*
*Carum carv*i (Caraway)	13.94±0.02 *RSD* 8.7% *(n=3)*
Coriandrum sativum (Coriander)	9.56±0.02 *RSD* 8.9% *(n=3)*

Figure 1. Free radical scavenging capacity of the extracts measured in DPPH assay.



As mentioned above, IC50% is the parameter representing the seeds' concentration capable to inhibite 50% of the amount of used DPPH.

It was determined by drawing a graph with simple concentration on the abscissa and free radical inhibition capacity IC(%) as ordinate. A series of samples were prepared as already described. The initial seed sample was diluted in a manner to obtain a linear graph with lines in the zone of 0 to 50% radical scavenging capacity. The sample concentration with reduces 50% of free radicals can be calculated by using a graph equation. A free radical scavenging activity was showed at all concentrations studied. It is evident that the 50% of inhibition value for *Foeniculum vulgare* (Fennel) methanol extract seems to be fairly significant when compared to the methanol extract of *Coriandrum sativum* (Coriander). IC50% methanolic extract of *Foeniculum vulgare* (Fennel) (113.19 mL/L) was necessary to obtain 50% of DPPH degradation. IC50% values of the extracts were compared to IC50% value of a "standart" antioxidant, in this case ascorbic acid (AA), obtained by the same procedure.

DISCUSSION

The systematic literature collection, pertaining to this investigation indicates that the plant phenolics constitute one of the major groups of compounds acting as primary antioxidant or free radical terminators (Moussa A.M. et al., 2011). Therefore, it is worthwhile to determine their total amount in plants chosen for the study. Flavonoids one of the most diverse and widespread group of natural compounds, are likely to be the most important natural phenolics due to their broad spectrum of chemical and biological activities, including antioxidant and free radical scavenging properties (Kahkonen M.P. et al., 1999; Moussa A.M. et al., 2011). Therefore the contents of flavonoids is also determined.

Plant materials rich in phenolics are increasingly being used in the food industry because they retard oxidative degradation of lipids and improve the quality and nutritional value of food (Kahkonen M.P. et al., 1999, Saeed N. et al., 2012). Phenolic compounds are considered secondary metabolites and these phytochemical compounds derived from phenylalanine and tyrosine occur ubiquitously in plants and are diversified (Naczk M., 2004; Saeed N. et al., 2012). The methanol extract exhibited the highest total phenolics content, whereas the contents obtained with residual aqueous fraction were much smaller that is in agreement with other reports (Ao C. et al., 2008; Saeed N. et al., 2012). Phenolic compounds of plants are also very important because their hydroxyl groups confer scavenging ability.

Phenolic compounds of plants fall into several categories; chief among these are the flavonoids which have potent antioxidant activities (Saeed N. et al., 2012, Nunes et al., 2012). Flavonoids are naturally occurring in plants and are thought to have positive effects on human health. Studies on flavonoidic derivatives have shown a wide range of antibacterial, antiviral, anti inflammatory, anticancer, and anti-allergic activities (Di Carbo G. et al., 1999; Montoro P. et al., 2005; Saeed N. et al., 2012). Flavonoids have been shown to be highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various free radicals (Bravo L., 1998; Saeed N. et al., 2012) implicated in several diseases. So comparable with the findings in the literature for other extracts of plant products (Sahreen S. et al., 2011; Saeed N. et al., 2012) our results suggested that phenolic acids and flavonoids may be the major contributors for the antioxidant activity as the EC50 values of radical scavenging activity of *Lamiaceae family* and the contents of phenolics or flavonoids exhibited significant correlation. However, non significant correlation was found in case of hydrogen peroxide radical scavenging. It is known that different phenolic compounds have different responses in the Folin-Ciocalteu method. Similarly the molecular antioxidant response of phenolic compounds varies remarkably, depending on their chemical structure (Satue-Gracia M.T. et al., 1997; Saeed N. et al., 2012). In addition, there may be some interference rising from other chemical components present in the extract, such as sugars or ascorbic acid (Singleton VLet al., 1965; Saeed N. et al., 2012).

Polyphenolic compounds have been found to protect erythrocytes from oxidative stress or increase their resistance to damage caused by oxidants (Kaviarasan S. et al., 2004; Asgary S. et al.,2005; Bisvas S. et al., 2005; Valente M.J. et al., 2011). They are able to act as antioxidants in a number of ways, mainly as reducing agents, hydrogen donors, singlet oxygen quenchers, and metal chelating agents (Rice-Evans C. et al., 1996; Cao G. et al., 1997).High correlation was reported between the antioxidant capacity and total phenol and flavonoides contents of plants (Silva E. et al., 2007; Tawaha K. et al., 2007). Beside antioxidant capacity, phenolic compounds exhibit a wide range of biological activities, including anti-carcinogenic, anti-inflammatory, anti-viral, anti-allergic, estrogenic, immune-stimulating agents, antiallergenic, anti-artherogenic, antiinflammatory, anti-microbial, antithrombotic, anti stress, anti hyperglycemia, cardioprotective and vasodilatory effects (Tawaha K. et al., 2007). It is well known that plant phenolics, in general are the highly effective free radical scavenging and antioxidants.

Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertbutylhydroquinone (TBHQ) have been used widely as antioxidants in foods, but concerns over the safety of use have led towards interest in natural antioxidants (Wanasundara UN and Shahidi F., 1998; Martinez-Valverde et al.

2002). These synthetic antioxidants are substituted phenolic compounds, and subsequently much of the research on natural antioxidants has also focused on phenolic compounds, in particular the flavonoids and hydroxycinnamic acids (Wanasundara U.N. and Shahidi F., 1998; Guptaa N. and al., 2011). The antioxidant activities of phenolic compounds extracted from different sources have been studied in several foods and food model systems [Wanasundara U.N. and Shahidi F., 1998].

The phytochemical screening and quantitative estimation of the percentage of chemical constituents of the plants studied showed that the dry seeds were rich in rutin and tannins. One of the bioactive flavonoids, rutin, is present in substantial amounts in various plants (Atanassova M. and Christova-Bagdassarian V., 2009; Guptaa N. and al., 2011). Rutin has desirable physiological and biological properties, such as anti-oxidation, anti-inflammation, anti-hypertension, vasoconstrictive, spasmolitic and a positive inotropic effect (Guptaa N. and al., 2011; Kuntirc V. et al., 2011; Landberg R. et al., 2011). Tannins are naturally occurring, high molecular weight polyphenols which can be divided into hydrolysable tannins and condensed tannins. Tannins are the most abundant antioxidants in the human diet and they exhibit many biologically important functions which include protection against oxidative stress and degenerative diseases (Atanassova M. and Christova-Bagdassarian V., 2009; Khomdram D. et al., 2011). The oxidation inhibiting activities of tannins have been known for a long time (Khomdram D. et al., 2011).

Several techniques have been used to determine the antioxidant activity *in vitro* in order to allow rapid screening of substances since substances that have low antioxidant activity *in vitro*, will probably show little activity *in vivo* (Naczk M. and Shahidi F., 2004; Saeed N. et al., 2012). Free radicals are known to play a definite role in a wide variety of pathological manifestations. Antioxidants fight against free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms (Umamaheswari M. and Chatterjee T.K., 2008; Saeed N. et al., 2012). The electron donation ability of natural products can be measured by 2,2-diphenyl-1- picrylhydrazyl radical (DPPH) purple-coloured solution bleaching (Naczk M. and Shahidi F., 2004; Saeed N. et al., 2012). The method is based on scavenging of DPPH through the addition of a radical species or antioxidant that decolourizes the DPPH solution. The degree of colour change is proportional to the concentration and potency of the antioxidants. A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound under test (Alves C.Q. et al., 2010; Saeed N. et al., 2012). Results of this study suggest that the plant extract contain phytochemical constituents that are capable of donating hydrogen to a free radical to scavenge the potential damage. Superoxide radical is considered a major biological source of reactive oxygen species (Meyer A.S. and Isaksen A., 1995; Saeed N. et al., 2012). Although superoxide anion is a weak oxidant, it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress (Krishnaiah D. et al., 2011; Saeed N. et al., 2012).

The extract showed high total phenol and flavonoid contents. Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources and show significant antioxidant activity (Ebrahimzadeh M.A. et al., 2010). DPPH is a stable nitrogencentred free radical, the colour of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron- donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers (Nabavi S.M. et al., 2008; Ebrahimzadeh M.A. et al., 2010;). The phenol and flavonoid contents of this plant may be responsible for its good DPPH-scavenging activity (Ebrahimzadeh M.A. et al., 2010). The correlation between total phenol contents and antioxidant activity has been widely studied in different foodstuffs such as fruit, vegetables and spices (Ghasemi K. et al., 2009; Nabavi S.M. et al., 2009; Ebrahimzadeh M.A. et al., 2010).

Phenolic compounds are ubiquitous in plants, and when plant foods are consumed, these phytochemicals contribute to the intake of natural antioxidants in the human diets. Agro-industrial by-products are good sources of phenolic compounds, and have been explored as a source of natural antioxidants (Balasundram N. et al., 2006). While the use of naturally occurring phenolic compounds as food antioxidants is particularly interesting, practical aspects that need to be considered include extraction efficiency, availability of sufficient raw material, and toxicity or safety considerations. The very complexity in the phenolic compounds profile of these by-products has to be resolved to obtain the optimum antioxidant efficiency (Balasundram N. et al., 2006).

CONCLUSIONS

In this paper an original data for total phenolic and total flavonoid contents are a basis for assessment of the role of Bulgarian plants (dry seeds) against free radicals effect and will enrich the national food composition database. The presented values for rutin and tannin contents are close to *Foeniculum vulgare* (Fennel), *Anethum graveolens* (Dill), *Pimpinella anisum* (Anise), *Carum carv*i (Caraway) and *Coriandrum sativum* (Coriander) but the comparison is not possible because of the different approach for analysis. Presence of all the above phenolic compounds in seeds

suggests that select species of family Apiaceae in methanolic extracts have the ability to provide protection against oxidation-linked diseases. Such seed ingredients in whole food form can be used as condiments with a range of food designs for better dietary management of hyperglycemia linked to type II diabetes.

REFERENCES

1. Albano S.M., Miguel M.G. (2011) Biological activities of extracts of plants grown in Portugal. *Industrial Crops and* Products, 33(3): 338–343.
2. Al-said M.S., Abdel Sattar E., El-Feraly F., Nahrstedt A., Coen M. (1996) New Sulfides from *Ferula rutabensis*. *Int J Pharmacolog*. **34**: 189-193.
3. Ao C., Li A., Elzaawely A.A., Xuan D.T., Twata S (2008) Evaluation of antioxidant and antibacterial activities of Ficus microcarpa L. fill extract. *Food Contrl*, **19**: 940–948.
4. Alves C.Q., David J.M., David J.P., Bahia M.V., Aguiar R.M (2010): Methods for determination of *in vitro* antioxidant activity for extracts and organic compounds. *Química Nova*, **33**: 2202–2210.
5 Arslan N., Gurbuz B., Sarihan E.O. (2004) Variation in Essential Oil Content and Composition in Turkish Anise (Pimpinella anisum L.) Populations. *Turk. J. Agric. Forest.*, **28**: 173-177.
6. Asgary S., Naderi G., Askari N. (2005) Protective effect of flavonoids against red blood cell hemolysis by free radicals. *Exp. Clin. Cardiol*., **10**: 88–90.
7. Atanassova M., Christova-Bagdassarian V. (2009) Rutin content in plant products. *JUCTM*, **44**(2): 201-203.
8. Atanassova M., Christova-Bagdassarian V. (2009) Determination of tannins content by titrimetric method for comparison of different plant species. *JUCTM*, **44**(4):4 13-415.
9. Balasundram N., Sundram K., Samman S. (2006) Analytical, Nutritional and Clinical Methods Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chem.*, **99**: 191–203.
10. Bektas T.L., Sihoglu-Tepe A., Daferera D., Polissiou M., Sokmen A. (2007) Chemical composition and antioxidant activity of the essential oil of Clinopodium vulgare. *Food Chemistry*, **103**: 766–770.
11. Biswas S., Bhattacharyya J., Dutta A.G. (2005) Oxidant induced injury of erythrocyte-role of green tea leaf and ascorbic acid. *Mol. Cell. Biochem.*, **276**: 205–210.
12. Bondet V., Brand-Williams W., Berset C. (1997) Kinetics and Mechanisms of Antioxidant Activity using the DPPH• Free Radical Method, *Lebensmittel-Wissenschaft und Technologie*, **30**: 609–615.
13. Bravo L: (1998) Polyphenols: chemistry, dietary sources, metabolism and nutritional significance. *Nutr Reviews*, **56**: 317–333.
14. Cao G., Sofic E., Prior R.L. (1997) Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. *Free Radic. Biol. Med.*, **22**: 749–760.
15. Di Carlo G., Mascolo N., Izzo A.A., Capasso F: (1999) Flavonoids: old and new aspects of a class of natural therapeutic drugs. *Life Sci* **65:** 337–353.
16. Ebrahimzadeh M.A., Nabavi S.F., Nabavi S.M., Eslami B. (2010) Antihypoxic and antioxidant activity of Hibiscus esculentus seeds. *Grasas y Aceites*, **61**(1): 30-36.
17. Ebrahimzadeh M.A., Nabavi S.M., Nabavi S.F., Eslami B., Rahmani Z. (2010) Antioxidant and Antihaemolytic Activities of the Leaves of *Kefe cumin* (Laser trilobum L) Umbelliferae**,** *Tropical J of Pharmac.* Res. **9** (5): 441-449.
18. Gezer K., Duru M.E., Kivrak I., Turkoglu A., Mercan N., Turkoglu H., Gulcan S. (2006). Free-radical scavenging capacity and antimicrobialactivity of wild edible mushroom from Turkey. *African .J. Biotechnol*., **5**(20):1924-1928.
19. Ghasemi K., Ghasemi Y., Ebrahimzadeh M.A. (2009) Antioxidant activity, phenol and flavonoid contents of 13 Citrus species peels and tissues. *Pak J Pharm Sci*, **22**(3): 277- 281.
20. Gilani A.H., Jabeen Q., Ghayur M.N., Janbaz K.H., Akhtar M.S. (2005) Studies on the antihypertensive, antispasmodic, bronchodilator and hepatoprotective activities of the carum copticum seed extract. *J. Ethnopharm.*, **98**: 127-135.
21. Guptaa N., Sharmaa S.K., Ranab J.C., Rajinder S. (2011) Chauhana Expression of flavonoid biosynthesis genes vis-a-vis rutin content variation in different growth stages of Fagopyrum species, *J. of Plant Physiol.*, **168**: 2117–2123.
22. Husain S.Z., Malik R.N., Javaid M., Bibi S. (2008) Ethonobotanical properties and uses of medicinal plants of morgah biodiversity park, *Rawalpindi. Pak. J. Bot.***40**: 1897-1911.
23. Iranshahi M., Amin G., Amini M., Shafiee A. (2003) Sulfur containing derivatives from *Ferula persica* var. *latisecta*. *Phytochemistry*., **63**: 965-966.
24. Iranshahi M., Amin G., Salehi-Sourmaghi M.H., Shafiee A., Hadjiakhoondi A. (2006) Sulphur-containing compounds in the essential oil of the root of *Ferula persica* Willd. var. persica. *Flav Fragr J.*, **21**:260-261.

25. Iranshahi M., Arfa P., Ramezani M., Jaafari M.R., Sadeghian H., Bassarello C. (2007) Sesquiterpene coumarins from *Ferula szowitsiana* and in vitro antileishmanial activity of 7-prenyloxycoumarins against promastigotes. *Phytochemistry*., **68**: 554-561.
26. Iranshahi M., Hassanzadeh-Khayyat M., Sahebkar A., Famili A. (2008) Chemical composition of the fruit oil of *Ferula flabelliloba*. *J Essent Oil-Bear Plants*.; **11**: 143-147.
27. Iranshahi M., Kalategi F., Rezaee R., Shahverdi A.R., Ito C., Furukawa H. (2008) Cancer chemopreventive activity of terpenoid coumarins from *Ferula* species. *Planta Med.* **74**: 147-150.
28. Iranshahi M, .Kalategi F., Sahebkar A., Sardashti A., Schneider B. (2010) New sesquiterpene coumarins from the roots of Ferula flabelliloba. *Pharm Biol*., **60**: 657-662.
29. Iranshahi M., Sahebkar A., Hosseini S.T., Takasaki M., Konoshima T., Tokuda H. (2010) Cancer chemopreventive activity of diversin from *Ferula diversivittata* in vitro and in vivo. *Phytomedicine*, **17**: 269-273.
30. Iranshahi M., Rezaee R., Sahebkar A., Bassarello C., Piacente S., Pizza C. (2009) Sesquiterpene coumarins from the fruits of *Ferula badrakema*. *Pharm Biol*. **47**: 344-347.
31. Iranshahi M., Shahverdi A.R., Mirjani R., Amin G.R., Shafiee A. (2004) Umbelliprenin from *Ferula persica* roots inhibits the red pigment production in Serratia marcescens. *Z Naturforsch*. **59**: 506-508.
32. Iranshahi M., Sahebkar A., Takasaki M., Konoshima T., Tokuda H. (2009) Cancer chemopreventive activity of the prenylated coumarin, umbelliprenin, in vivo. *Eur J Cancer Prev.*, **18**: 412-415.
33. Iranshahi M., Yazdi M.C., Hassanzadeh-Khayyat M., Sahebkar A. (2009) Sulfur containing compounds in the volatile oil of *Ferula latisecta Rech. f.* & *Aell*. Leaves. *J Essent Oil-Bear Plants.*, **12**: 64-68.
34. Kahkonen M.P., Hopia A.I., Vuorela H.J., Rauha J.P., Kujala T.S. (1999) Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. and Food Chem.*, **47**: 3954-3962.
35. Kaviarasan S., Vijayalakshmi K., Anuradha C.(2004) Polyphenol-rich extract of fenugreek seeds protect erythrocytes from oxidative damage. *Plant Foods Hum. Nutr.*, **59**: 143–147.
36. Khomdram D., Singh P.K. (2011) Polyphenolic Compounds and Free Radical Scavenging Activity in Eight *Lamiaceae* Herbs of Manipur *Notulae Scientia Biologicae*, **3**(2):108-113.
37. Kiselova Y., Ivanova D., Chervenkov T., Gerova D., Galunska B., Yankova T. (2006) Correlation between the in vitro antioxidant activity and polyphenol content of aqueous extracts from Bulgarian herbs. *Phytoth. Res*., **20**: 961-965.
38. Kogure K., Yamauchi I., Tokumura A., Kondou K., Tanaka N., Takaishi Y. (2004) Novel antioxidants isolated from plants of the genera *Ferula*, *Inula*, *Prangos* and *Rheum* collected in Uzbekistan. *Phytomedicine*, **11**: 645-651.
39. Krishnaiah D., Sarbatly R., Nithyanandam R.R: (2011) A review of the antioxidant potential of medicinal plant species. *Food Bioprod Process*, **89**: 217–233.
40. Kuntič V., Filipovič I., Vujič Z. (2011) Effects of rutin and hesperidin and their Al (III) and Cu (II) complexes on in vitro plasma coagulation assays. *Molecules*; **16**(2): 1378–1388.
41. Kwon Y.I., Vattem D.V., Shetty K. (2006) Evaluation of clonal herbs of Lamiaceae species for management of diabetes and hypertension. *Asia Pac. J. Clin. Nutr*., **15**: 107–118.
42. Landberg R., Sun Q., Rimm E.B., Cassidy A., Scalbert A., Mantzoros C.S., Hu F.B., van Dam R.M. (2011) Selected dietary flavonoids are associated with markers of inflammation and endothelial dysfunction in U.S. women. *J Nutr.*, **141**(4):618-625.
43. Lans C., Turner N., Khan T., Brauer G., Boepple W. (2007) Ethnoveterinary medicines used for ruminants in British Columbia, Canada. *J. Ethnobiol. Ethnomed.*, **3**: 11.
44. Lemhadri A., Hajji L., Michel J.B., Eddouks M. (2006) Cholesterol and triglycerides lowering activities of caraway fruits in normal and streptozotocin diabetic rats. *J. Ethnopharmacol*., **106**: 321-326.
45. Marinova D., Ribarova F., Atanassova M. (2005) Total phenolics and total flavonoids in Bulgarian fruits and vegetables. *JUCTM*, 4**0**(3): 255-260.
46. Martínez-Valverde I., Periago M.J., Provan G., Chesson A. (2002) Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (Lycopersicum esculentum). *J. Sci.Food Agric.*, **82**: 323–330.
47. Meyer A.S., Isaksen A. (1995) Application of enzymes as food antioxidants. Trends *Food Sci Tech*, 1995(6): 300–304.
48. Montoro P., Braca A., Pizza C., De Tommasi N. (2005) Structure-antioxidant activity relationships of flavonoids isolated from different plant species. *Food Chem*, **92**: 349–355.
49. Motai T., Daikonya A., Kitanaka S. (2004) Sesquiterpene Coumarins from *Ferula fukanensis* and nitric oxide production inhibitory effects. *J Nat Prod*., **67**: 432-436.
50. Moussa A.M., Emam A.M., Diab Y.M., Mahmoud M.E. and Mahmoud A.S (2011) Evaluation of antioxidant potential of 124 Egyptian plants with emphasis on the action of *Punica granatum* leaf extract on rats, International *Food Research J.*, **18**: 535-542.

51. Mozaffarian V. (1983), The Family of Umbelliferae in Iran- Keys and Distribution. Tehran:Research Institute of Forests and Rangelands Press;114-116.
52. Mozaffarian V. A. (1896) Dictionary of Iranian Plant Names. Tehran:Farhang-e Moaser 228-230.
53. Murray R.D.H, Mendez J., Brown S.A. (1982) The Natural Coumarins. 1st ed. New York:John Wiley & Sons Inc; 555-559.
54. Nabavi S.M., Ebrahimzadeh M.A., Nabavi S.F., Jafari M. (2008) Free radical scavenging activity and antioxidant capacity of Eryngium caucasicum Traut and Froriepia subpinnata. *Pharmacol. online*, **3**: 19-25.
55. Nabavi S.M., Ebrahimzadeh M.A., Nabavi S.F., Fazelian M., Eslami B. (2009) *In vitro* antioxidant and free radical scavenging activity of Diospyros lotus and Pyrus boissieriana growing in *Iran*. *Pharmacognosy Magazine*, **4**(18): 123-127.
56. Naczk M., Shahidi F. (2004) Extraction and analysis of phenolics in food. *J Chromatogr A*, **1054**: 95–111.
57. Nunes P.X., Silva S.F., Guedes R.J., Almeida S (2012) Biological oxidations and antioxidant activity of natural products, Phytochemicals as nutraceuticals - Global Approaches to Their Role in Nutrition and Health. 2012.
58. Oktay M., In I.G., Iu O.K. (2003) Determination of in vitro antioxidant activity of fennel (Foeniculum vulgare) seed extracts. *Lebensmittel-Wissenschaft und-Technol.*, **36**: 263-271.
59. Pimenov M.G., Leonov M.V. (1993) The genera of the Umbelliferae. Kew:*Royal Botanic Gardens*., 156.
60. Rajanikanth B., Ravindranath B., Shankaranarayana M.L. (1984) Volatile Polysulphides of Assafoetida. *Phytochemistry*., **23**: 899-900.
61. Rice-Evans C., Miller N., Paganga G. (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Rad. Biol. Med.*, **20**: 933–956.
62. Saeed N., Khan M.R. and Shabbir M. (2012) Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L, BMC *Complementary and Alternative Medicine*, **12**: 221.
63. Sahebkar A., Iranshahi M. (2010) Biological activities of essential oils from the genus *Ferula* (Apiaceae), *Asian Biomedicine*, **4**(6): 835-847.
64. Sahebkar A., Hassanzadeh-Khayyat M., Iranshahi M. (2010) Qualitative analysis of the hydro-distilled essential oil of *Ferula latisecta Rech. f.* & *Aell*. Roots from Iran. *J Essent Oil-Bear Plants.*, **13**: 340-346.
65. Sahreen S., Khan M.R., Khan R.A: (2011) Phenolic compounds and antioxidant activities of Rumex hastatus D. Don. Leaves. *J Med Plants Res*, **5**: 2755–2765.
66. Sallem F. (2010) Anti-diabetic potentials of phenolic enriched Chilean potato and select herbs of Apiaceae and Lamiaceae Families, A Thesis Presented by Submitted to the Graduate School of the University of Massachusetts Amherst in partial fulfillment of the requirements for the degree of Master of Science, 110.
67. Satue-Gracia M.T., Heinonen M., Frankel E.N (1997) Antioxidant activity of anthocyanin in LDL and lecithin liposome systems. *J Agric Food Chem* **45**: 3362–3367.
68. Shetty K., Wahlqvist M.L. (2004) A model for the role of the proline-linked pentose-phosphate pathway in phenolic phytochemical bio-synthesis and mechanism of action for human health and environmental applications. *Asia Pac J Clin Nutr*. **13**(1): 1-24.
69. Silva E., Souza J., Rogez H., Rees J., Larondelle Y. (2007) Antioxidant activities and polyphenolic contents of fifteen selected plant species from the Amazonian region. *Food Chem.*, **101**: 1012-1018.
70. Singh V., Jain D.K. (2007) Taxonomy of angiosperms. 6th ed. *India:Rastogi Publications*; 2007: 298.
71. Singleton V.L., Rossi J.A: (1965) Colourimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic*, **16**: 144–158.
72. Tamemoto K., Takais Y., Chen B., Kawazoe K., Shibata H., Higuti T. (2001) Sesquiterpenoids from the fruits of *Ferula kuhistanica* and antibacterial activity of the constituents of *F. kuhistanica*. *Phytochemistry*, **58**: 763-767.
73. Tawaha K., Alali F., Gharaibeh M., Mohammad M., El-Elimat T. (2007) Antioxidant activity and total phenolic content of selected Jordanian species. *Food Chem.*, 104: 1372-1378.
74. Umamaheswari M., Chatterjee T.K. (2008) In vitro antioxidant activities of the fractions of Coccinnia grandis L. leaf extract. *Afr J Trad Compl Altern Med*, **5**: 61–73.
75. Valente M.J., Baltazar A.F, Henrique R., Estevinho L., Carvalho M., (2011) Biological activities of Portuguese propolis: Protection against free radical-induced erythrocyte damage and inhibition of human renal cancer cell growth *in vitro*. *Food Chem. Toxicol*., **49**: 86–92.
76. Wanasundara U.N., Shahidi F. (1998) Antioxidant and prooxidant activity of green tea extracts in marine oils. *Food Chem.*, **63**: 335–342.
77. Wojdyło A., Oszmianrski J., Czemerys R. (2007) Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chemistry*, **105**: 940-949.
78. Yen G.C., Duh P.D. (1994) Scavenging effect of methanolic extracts of peanut hulls on free radical and active oxygen species. *J. Agr. Food Chem.* **42**: 629-632.

79. Zheng W., Wang S.Y. (2001) Antioxidant activity and phenolic compounds in selected herbs. *J. Agr. Food Chem.* **49:** 5165-5170.
80. Zhi-da M., Qi-fi M., Mizuno M., Tanaka T., Iinuma M. (1987) Polysulfanes in the volatile oil of *Ferula* species. *Planta Med.*, 53: 300-302.