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SCREENING OF TOTAL POLY PHENOLS AND VITAMIN E AS NATURAL ANTIOXIDANTS POTENTIAL FROM IRANIAN TRADITIONAL FOOD PLANTS EXTRACTS

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Abstract

Antioxidants are vital substances, which possess the ability to protect the body from damages caused by free radical-induced oxidative stress. The main objective of this study was to assess the antioxidant levels of a number of fruits and vegetables. We investigated the total phenolic contents in the plant foods used as traditional vegetables and fruits and well-known as sources of antioxidant using standard assay. Total phenolic contents ranged from 0.87 to 7.02mg gallic acid/g Dw in *Alocaccia indica* and *Solanum indicum,* respectively. Results showed that *portulaca* contains the most value (11.6 mg/100g),leaves of this plant may be considered as a potential new source of natural α- tocopherol, but vitamin E in *Solanum, Chlorophytum* and *Alocacia* are not detected. The plant foods possess valuable antioxidant properties for culinary and possible nutritive use.

Key Words: Antioxidant compound, Total phenolic content, Vitamin E, Plant foods.

Introduction

Phytochemicals exerting antioxidant actions are largely being recognized as of benefiting human health and disease prevention. These benefits may be a result of concerted actions of well-known antioxidants such as vitamin C, vitamin E and β -carotene. Indeed, phenolic compounds are ubiquitously distributed in the plant kingdom and exhibit a wide range of medicinal properties, including anti-inflammatory, anti-carcinogenic viral, anti-allergic and immune-stimulating agents (Larson, 1988). These protective effects have been mostly ascribed to their free radical scavenging, metal chelating and chain breaking effects. Many literature reports show a relatively strong correlation between the total phenolic content and the antioxidant capacity of plant extracts (Zheng, 2001; Cai, 2004; Tawaha 2007).

Crude extracts of fruits, herbs, vegetables, cereals, and other plant materials rich in phenolics are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions (Souri, 2008). The antioxidative effect is mainly due to phenolic components, such as flavonoids (Al-Farsi, 2007), phenolic acids, and phenolic diterpenes. Typical phenolics that possess antioxidant activity have been characterized as phenolic acids and flavonoids. Phenolic acids have repeatedly been implicated as natural antioxidants in fruits, vegetables, and other plants. Rosmarinic acid, an important phytochemical, has been found to be a potent active substance against human immunodeficiency virus type 1 (HIV-1) (Byers (1995).

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Among the fat-soluble vitamins, vitamin E is probably the most familiar and at the same time the most likely to be misused. Vitamin E was recognized in 1922 by Evans and Bishop as a dietary factor from plants that was essential for normal reproduction in rats. It was not until 1933 that it was identified as a group of substances known as tocopherols and tocotrienols and established as a dietary essential for humans. Vitamin E is a mixture of at least eight naturally occurring tocopherols and tocotrienols, each of which makes a different contribution to the vitamin E content in a food. It is generally agreed that the main function of vitamin E is as an antioxidant, or substance that prevents oxidation of other nutrients in food and body. Vitamin E also plays an important role in protecting vitamin A, vitamin C, and unsaturated fatty acids in the food against undesirable changes as the result of oxidation. The study of the role of vitamin E as an antioxidant is complicated by the fact that other substances, such as the mineral selenium, also function as biological antioxidants and perform some but not all of the roles of vitamin E. Fruits and vegetables provide about 10% of the remaining vitamin E in the diet; the rest comes from all the other food groups, with each providing relatively small amounts. Tocopherol is relatively stable at normal cooking temperatures. Foods stored at freezing temperatures lose an appreciable amount of their vitamin E content unless the temperatures are very low to prevent any oxidative changes. Vitamin e is also destroyed by exposure to light and oxygen (Pokorny, 2001; Nicoli 1997). Present study attempted extraction of phenolics and vitamin E contents of some plant foods as new potential sources of natural antioxidants.

Materials and Methods

Three species of fruits and vegetables viz Alocacia indica Sch, Eulophia Ochreata Lindl ,, Momordica dioicia Roxb., were purchased from were collected from three various localities of Maharashtra, India. Five wild edible plants viz Asparagus officinalis, Chlorophytum comosum, Codia myxa, Portulaca oleracia and Solanum indicum were collected from three areas in around Behbahan, Iran in April 2008. Fresh fruits and vegetables were cleaned with water and external moisture wiped out with a dry cloth. The edible portion of the individual fruits was separated, dried in a hot air oven at 50°C for 1 hr. The dried samples were powdered in blender for further study. Some of the plants such as Asparagus officinalis, Chlorophytum comosum,and Portulaca oleracia dried under shade so as to prevent the decomposition of chemical compounds. The Plant foods analysis was carried out in Lab. of Department of Food Science, Ramin Agricultural University, Ahvaz, Iran.

Chemical reagents

The chemical reagent ABTS [2,20-Azino-bis (3-ethylbenzthiazoline- 6-sulfonic acid)] was purchased from CALBIOCHEM (Darmst adt, Germany). All other chemicals used were of analytical and HPLC grade and obtained from Sigma Co. (St. Louis, MO).

Phenolics analysis

Dried plants (50 g) were crushed using a laboratory mill. Ground dry plant material (500 mg) was weighed into a test tube and 2 ml of a mixture of enzymes (5 mg of each enzyme: b-glucosidase, b xylosidase, b-galactosidase, and b-hesperidinase) and 0.5 ml Sulfatase type H-2 diluted in citrate buffer at pH 5.5 were added. Moreover, SO2 (from NaHSO3), in order to prevent oxidative losses of phenolics, was added. The tested sample with enzyme were hydrolyzed in a water bath for 1 h at 37 ºC. Then, samples were chilled to 20 ºC and kept in this condition for 24 h. Then 2 ml of methanol were added to each vial and sonificated for 10 min by shaking occasionally (BAS-10, Poland). Then, samples were centrifuged (5 min, 19000g; MPW- 250, Poland) and the clear supernatant was injected into the HPLC equipment. 2.4. Identification and quantification of phenolic compounds Twenty-microliter samples of each supernatant of spices were analyzed using an HPLC system equipped with an L-7100 pump (Merck Hitachi) and an L-7455 photodiode array UV–VIS detector (Merck Hitachi). The samples were injected using an L-7200 autosampler (Merck Hitachi). The polyphenols were separated using a LiChroCART_ 125-3 Purospher_ RP-18 (5 lm) MerckLabs column heated at 30 ºC (L-7350 Merck Hitachi). The mobile phase was composed of solvent A (4.5% formic acid) and solvent B (80% of acetonitrile and 20% of solvent A). The programme began with isocratic elution with 95% A (0–1 min); then a linear gradient was

used until 16 min, lowering A to 20%; from 17 min to 24 min A decreased to 0%. The flow rate was 1 ml min_1, and the runs were integrated at 280 and 320, 360 nm for hydroxycinnamic acid and flavonoid derivatives, respectively. Scanning was performed from 200 to 600 nm. Phenolic compounds were identified by comparing retention times and UV–VIS spectra with those of pure standards to indicate the preparations of standards and the range of calibration curves. The repeatability of the quantitative analysis
was ±4%. The analyses were replicated (n = 3), and the contents given as mean values, plus or minus the standard deviation. The results were expressed as milligrammes of each compound per g of dry weight (dw) spices.

Determination of Vitamin E

This evaluation was done by method of Sanchez-Machado with a little modification. A sub sample of 0.40 g(±0.001g) was weighed out in a screw-top assay tube. Two hundred microliters of pyrocatechol solution was used as an antioxidant. Five milliliters of KOH solution (0.5 M in methanol) wert added and immediately vortexed for 20 Sec. The tubes were placed in a water bath at 80ºC for 15 min, removed every 5 min, and vortexed again for 15 Sec. After cooling in iced water, 1 ml of distilled water and 5ml of hexane was added, and the mixture was rapidly vortexed for 1 min, then centrifuged for 2 min. Three milliliters of the upper phase were transferred to another test tube and dried under nitrogen. The residue was redissolved in 3 ml of the HPLC mobile phase(68:28:4(v/v/v)methanol:acetonitrile:water), then membrane-filtered(poresize0.50µ; Whatman, Clifton, New Jersey, USA). Finally, an aliquot of 20 µl was injected into the HPLC column. Before injection, the extracts were maintained at -10 ºC away from light.Stock standard solution of α-tocopherol(0.5 mg/ml) prepared also in 100% methanol and stored at -10 ºC away from light.

Statistical analysis

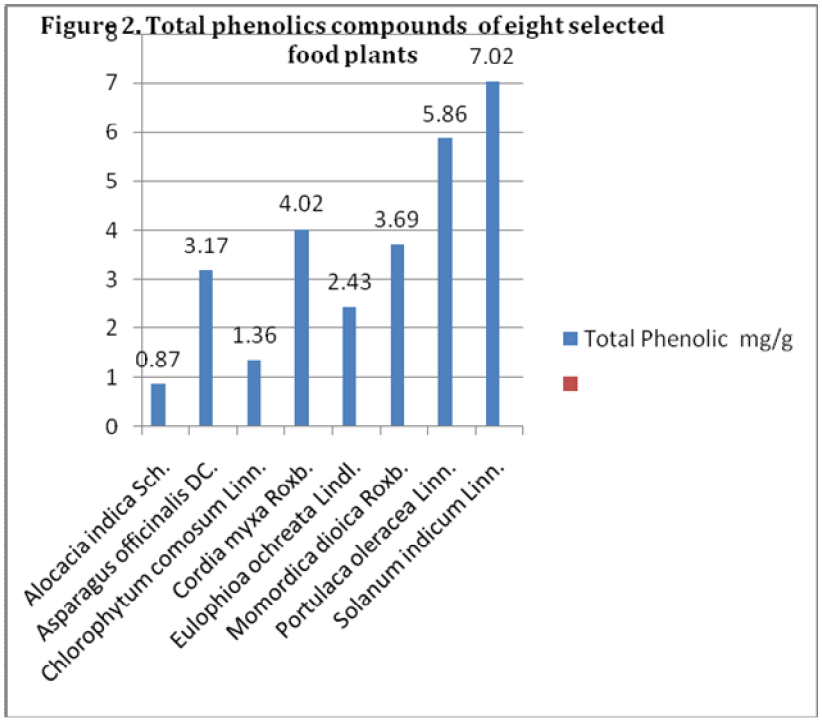
Three replicates of each sample were used for statistical analysis. Data were subjected to analysis of variance, and means were compared by least significant difference (LSD). Differences at $P<0.05$ were considered to be significant.

Results and Discussion

Figure 2 showed amounts of total phenolics compounds of selected some food plants. *S. indicum* with (**7.02**±2.1mg/g) highest value and *A. indica* with (0.87±2.1 mg/g) lowest value respectively. The ranking order of eight plant species from point of view of phenolic compounds amounts was as follows: *S. indicum* > *P.oleracia* > *C.myxa* > *M.dioica* > *A. officinalis* > *E. ocherata*> *C. comocum*> *A. indica*. Results showed that total phenolic amounts of *Momordica dioicia Roxb* and of *Cordia myxa Roxb*were comparable with total phenolic amount of Mint vegetable. but the amounts were more than total phenolic amounts of other vegetables (Vinson, 2005). Total phenolic amount of *Solanum indicum Linn* was more than total phenolic amounts of Black berry Stratil (2007) and Cranberry Vasco (2008). Therefore, antioxidant capacity of *Solanum indicum Linn*. was high and antioxidant capacity of *Alocacia indica Sch*. was low. Phenolic compounds could be a major determinant of antioxidant potentials of food plants and could therefore be a natural source of antioxidants and because Phenolic compounds have been associated with the health benefits derived from consuming high levels of fruits and vegetables, Therefore, *Solanum indicum Linn*. has high preservation capacity and nutritional values, because total phenolic compounds prevent from damage of nutrients contain double bonds such fatty acids, flavor compounds even proteins and amino acids and other compounds Kahkonen (1999).

The amount of total phenolics varied in different plant foods and ranged from 0.87 to 7.02 mg GAE/g of dry material. The amount of total phenolic compounds in all tested plant foods was higher than the other Lamiaceous plants reported such as Thymus vulgaris Kaur (2002), Mentha piperita, Melissa officinalis and Rosmarinus officinalis (Lister , 2001). Some selected phenolics of

these plant foods, have previously been separated and identified by comparison with authentic standards using reversed-phase high performance liquid chromatography (HPLC), and rosmarinic acid was the predominant phenolic acid in these plant foods (Mazumder, 1997). Many factors could contribute to this variation, such as the plant variety, growing condition, maturity, season, geographic location, soil type, storage conditions and amount of sunlight received. Other contributing factor for this difference may be also due to sample preparation and analytical procedures (Pietta, 1998).



Data are displayed from mean values with three repeat ± SD

More than 4000 phenol compounds (flavonoids, monophenols and polyphenols) are found in vascular plants. Phenolic compounds, such as querecetin, rutin, narigin, catechine, caffeic acid, gallic acid and chlorogenic acid are very important plant constituents. The type of phenolic compounds is an important factor since following consumption of fruits, phenolics are usually present in plasma at concentration not exceeding 10 M. There are several factors involves in determining the ratio of free to conjugated phenolics in plasma. Among these factors are the type of polyphenol, the fruit stage of maturity, the state of health of the fruit, method of harvesting and storage (Sellappan, 2002).

Yet, current data in the literature on the relationship between the polyphenol content of plants and their antioxidant activity are sometimes contradictory. While some authors have observed such a high correlation between the two others found no such correlation exists or only a very weak one (Spanos, 1990).

Samples vitamin E amounts were compared, It is observed that *portulaca* contains the most value (11.6 mg/100g), but vitamin E in *Solanum, Chlorophytum* and *Alocacia* are not detected (Figure 1). *Portulaca* contains most values of phenolic compounds and vitamin E, therefore this plant have the highest of antioxidant property. The antioxidant property give to plant high shelf-life then high consumption capacity in between of people, therefore this plant have high nutritional value from point of view of vitamin E. Vitamin E is actually a collective term for eight compounds: (α-,β-,γ-,δ) tocopherols, and (α-,β-,γ-,δ) tocotrienols, but α- tocopherol, accounts for 90% of endogenous vitaminE activity in humans. Vitamin E is readily incorporated into cell membranes, which, being rich in polyunsaturated fatty acids, are highly susceptible to damage by free radicals derived from metabolic activity. Epidemiological studies show a strong inverse correlation between risk of cancer and vitamin E intake at the population level, but the association is not corroborated by studies of individual taking supplements. Moreover, a well –controlled investigation designed to

test the hypothesis that dietary supplementation with vitamin E would reduce the recurrence of adenomas in patients who had undergone polypectomy showed no evidence of a protective effect. Similarly, a prolonged placebo- controlled intervention with vitamin E or vitamin E and beta-carotene failed to prevent the development of long cancer in smokers (Sun Chu & Wu Lia, 2002; Zheng, 2001; Simon, 1999).

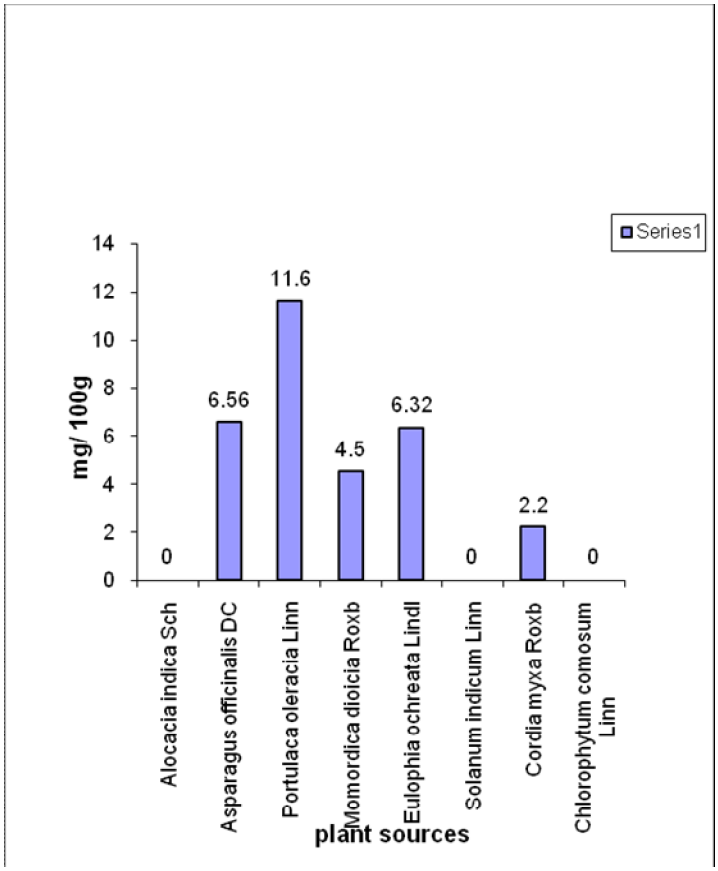


Figure1. Amount of Vitamin E of eight edible plants obtained from India and Iran.

Data are displayed from mean values with three repeat

Food sources of phenolic compounds

Though phenolic compounds are present in almost all foods of plant origin, fruits, vegetables, and beverages are the major sources of these compounds in the human diet (Hertog, 1993).

Fruits and vegetables

There are wide variations between the total phenolics contents of the different fruits or vegetables, or even for the same fruits or vegetables reported by different authors. These differences may be due to the complexity of these groups of compounds, and the methods of extraction and analysis (Bravo, 1998; Kalt, 2001). For example, phenolic compounds present in fruits are found in both free and bound forms (mainly as b-glycosides), but as the latter are often excluded from analyses, the total phenolics contents of fruits are often underestimated (Sun, 2002). Besides, phenolics contents of plant foods depend on a number of intrinsic (genus, species, cultivars) and extrinsic (agronomic, environmental, handling and storage) factors (Tomas Berberan, 2001). Species differences are also pronounced, which suggests that the phenolics content of some fruits, i.e., banana, litchi (lichee), mango, and persimmon is considerably lower than that of berries and grapes. Asami, Hong, Barrett, and Mitchell (Asami, 2003) reported that organically grown strawberries were found to have higher phenolics content than conventionally grown crops, though another study could not establish such a correlation (Hakkinen & Torrenen, 2000). Processing and

storage may have varying impacts on different phenolic compounds, as seen in berry processing where myricetin and kaempferol were found to be more prone to losses than quercetin (Hakkinen, 2000).
Obtained results from phenolics contents (per mg/g) of this study was more than shown results in Table 2.

Table 2. Phenolics content of selected vegetables

Vegetable	Total phenolics Content	Reference
Broccoli	101.6 ± 1.24a	Chu et al. (2002)
Brussel sprouts	68.8± 1.3b	Kaur and Kapoor (2002)
Cabbage	54.6 ±7.0a	Chu et al. (2002)
Carrot	56.4 ±5.1a	Chu et al. (2002)
Cucumber	19.5 ±1.6a	Chu et al. (2002)
Mint	399.8 ± 3.2b	Kaur and Kapoor (2002)
Spinach	91.0 ±8.5a	Chu et al. (2002)
Tomato	25.9 ±50.0c	Martı´nez-Valverde et al. (2002)

a mg gallic acid equivalents/ L.
b mg ferulic acid equivalents/L.

Conclusions

The total phenolic content of eight selected wild plants were examined. These plants showed different total phenolic contents. In particular, the stem and tuber of *A. Officinalis* and *E. ocharata* respectively(vitamin E antioxidant) and the leaves of *P. Oleracia* possessed the highest antioxidant amounts(both vitamin E and phenolics antioxidants) and thus could be potential rich sources of natural antioxidants. Antioxidants in these plants were capable of reducing oxidants and scavenging free radicals. In the future, the specific compounds with high antioxidant capacities should be isolated, purified and identified from these plants to further develop natural antioxidants.

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