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ScienceDirect
Food Chemistry 104 (2007) 364–368
Food Chemistry
www.elsevier.com/locate/foodchem

Comparative evaluation of the antioxidant potential of some Iranian medicinal plants

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Received 11 May 2006; received in revised form 27 September 2006; accepted 22 November 2006

Abstract

Medicinal plants are a source for a wide variety of natural antioxidants. In the study reported here, we have conducted a comparative study between five medicinal plants having the same geographic origin: the Hamadan region in the west of Iran and growing in the same natural conditions. The amount of total phenolics and total flavonoids for parts of these plants used in Iranian popular medicine were evaluated. Furthermore, antioxidant activities for these parts using vitamin C equivalent antioxidant capacity (VCEAC) test were also evaluated. The results show that the antioxidant activities varied greatly among the different plant parts used in this study and some plants are rich in natural antioxidants especially leaves of *Lavandula officinalis* and of *Melissa officinalis*. A positive correlation between total phenolic or flavonoid contents and VCEAC was found with a correlation coefficient of $R^2 = 0.961$ and $R^2 = 0.817$, respectively. These findings show that phenolics in these plants provide substantial antioxidant activity.

Keywords: Medicinal plants; Phenolics; Flavonoids; Vitamin C equivalent antioxidant capacity (VCEAC)

1. Introduction

Oxidative stress is mediated by reactive oxygen species (ROS) which are generated during the normal and aberrant cellular metabolism that utilizes molecular oxygen. The imbalance between production of ROS like $O_2^{\cdot-}$, H_2O_2, $OH^{\cdot}$, $ROO^{\cdot}$ and the capacity of the normal detoxification systems in favour of the oxidants leads to oxidative stress, which itself leads to cellular damage caused by the interaction of ROS with cellular constituents. Oxidative stress is involved in many acute and chronic diseases including cancer, cardiovascular troubles and neurodegenerative diseases. The balance between antioxidation and oxidation is believed to be critical in maintaining a healthy biological system (Hong & Liu, 2004; Judge, Jang, Smith, Hagen, & Leeuwenburgh, 2005; Katalinic, Milos, Kulisic, & Jukic, 2006; Montuschi, Barnes, & Roberts, 2004; Pak et al., 2006).

Recently, many researchers have taken a great interest in medicinal plants for their phenolic concentrations and related total antioxidant potential (Djeridane et al., 2006; Katalinic et al., 2006; Wong, Li, Cheng, & Chen, 2006). It is reported that some medicinal plants contain a wide variety of natural antioxidants, such as phenolic acids, flavonoids and tannins, which possess more potent antioxidant activity than dietary plants (Wong et al., 2006). Many investigations indicate that these compounds are of great value in preventing the onset and/or progression of many human diseases (Halliwell & Gutteridge, 1989; Halliwell, Gutteridge, & Cross, 1992). The health-promoting effect of antioxidants from plants is thought to arise from their protective effects by counteracting reactive oxygen species (ROS) (Wong et al., 2006).

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doi:10.1016/j.foodchem.2006.11.069

The purposes of this study were to determine the content of total phenolics and total flavonoids and to evaluate total antioxidant activity of five Iranian medicinal plants using the vitamin C equivalent antioxidant capacity (VCEAC) test.

2. Materials and methods

2.1. Chemicals

Aluminium chloride ($AlCl_3$), Catechin and Gallic acid were purchased from Acros Organics. Ascorbic acid, 2-2′-azino-bis(3ethylbenzothiazoline-6-sulfonic acid)diammonium salt (ABTS), PBS buffer, 2-2′-azobis(2methylpropionamidine)dichloride (AAPH), Folin–Ciocalteu's phenol reagent, Sodium carbonate (Na_2CO_3) and Sodium nitrite ($NaNO_2$) were purchased from Sigma Chemical Company.

2.2. Plant material and protocol

Five medicinal plants were collected from the Hamadan region in the west of Iran. Plant parts have been chosen in relation to Iranian popular medicine use: flowers of *Alcea kurdica* (Malvaceae), flowers of *Stachys lavandulifolium* (Lamiaceae), root of *Valeriana officinalis* (Valerianaceae), leaves of *Lavandula officinalis* (Lamiaceae) and leaves of *Melissa officinalis* (Lamiaceae).

Contrary to the other plants, *A. kurdica* and *S. lavandulifolium* are endemic flora of the Hamadan region.

The extractions were carried out using the same protocol. Grinded plant parts macerated in pure water for 12 h at room temperature, and then for 12 h at 37 °C temperature. Afterwards the filtrate was lyophilised.

2.3. Determination of total phenolics

Total phenolic contents were evaluated with Folin–Ciocalteu's phenol reagent (Kim, Chun, Kim, Moon, & Lee, 2003) using spectrophotometric analysis (Cary 50 Scan UV–Visible apparatus). Briefly, an aliquot (1 ml) of standard solutions of gallic acid at different concentrations or appropriately diluted extracts was added to a 25 ml volumetric flask containing 9 ml of ddH_2O. A reagent blank using ddH_2O was prepared. One milliliter of Folin and Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was added with mixing. The solution was then immediately diluted to volume (25 ml) with ddH_2O and mixed thoroughly. After incubation for 90 min at 23 °C, the absorbance versus prepared blank was read at 750 nm. Total phenolic contents in medicinal plants were expressed as mg gallic acid equivalents (GAE)/g dry weight. Samples were analyzed in three replications.

2.4. Determination of total flavonoids

Total flavonoid contents were measured according to a colorimetric assay (Zhishen, Mengcheng, & Jianming, 1999). A 1 ml aliquot of standard solutions of catechin at different concentrations or appropriately diluted samples was added to a 10 ml volumetric flask containing 4 ml ddH_2O. At zero time, 0.3 ml 5% $NaNO_2$ was added to the flask. After 5 min, 0.3 ml 10% $AlCl_3$ was added. At 6 min, 2 ml of 1 M NaOH was added to the mixture. Immediately, the solution was diluted to volume (10 ml) with ddH_2O and mixed thoroughly. Absorbance of the mixture, pink in colour, was determined at 510 nm versus the prepared blank. Total flavonoid contents in medicinal plants were expressed as mg catechin equivalents (CE)/g dry weight (dw). Samples were analyzed in three replications.

2.5. Determination of total antioxidant activity using ABTS radical scavenging capacity assay

VCEAC test developed by Kim, Chun, et al. (2003) was used in this study. Total antioxidant activities of medicinal plants were determined by scavenging blue–green ABTS radicals and were expressed as mg vitamin C equivalent (VCE) per g dry weight.

Briefly, 1 mM AAPH, a radical initiator, was mixed with 2.5 mM ABTS in phosphate-buffered saline (PBS, pH 7.4). The mixed solution was heated in a water bath at 68 °C for 13 min. The resulting blue–green ABTS radical solution was adjusted to the absorbance of 0.650 ± 0.020 at 734 nm with additional PBS. Twenty microliters of sample was added to 980 μl of the ABTS radical solution. The mixture was incubated in a 37 °C water bath under restricted light for 10 min. The control consisted of 20 μl 50% methanol and 980 μl of ABTS radical solution. The decrease of absorbance at 734 nm was measured 10 min later. Samples were analyzed in three replications.

2.6. Statistical analysis

Data were reported as mean ± standard deviation. To examine antioxidant activity differences between extracts, we have used ANOVA followed by PLSD of Fisher test. For all statistical comparisons, the level of significance was set at $p < 0.05$. All statistical analyses were carried out using the Statview® 4.5 statistical package (Abacus Concepts, Inc.).

3. Results

3.1. Determination of total phenolics

Table 1 shows the traditional uses of some plants in Iranian society and their total phenolic contents, which varied between 2.15 and 20.3 mg of GAE/g dw. The highest concentration of total phenolics was observed in leaves of *M. officinalis*, followed by leaves of *L. officinalis*, flowers of *S. lavandulifolium* and root of *V. officinalis*. The flowers of *A. kurdica* had the lowest phenolics concentration.

Table 1
Medicinal uses of some Iranian plant parts and their amounts of total phenolics

Scientific name	Plant parts used in Iranian popular medicine and their effects and/or uses	Total phenolics (mg GAE/g dw)
A. kurdica	Flowers: inflammation and cough (Piri et al., 2006)	2.15 ± 0.1
V. officinalis	Root: depression (Fintelmann & Weiss, 2004)	6.31 ± 0.14
S. lavandulifolium	Flowers: depression (Rabbani et al., 2003; Rabbani et al., 2005)	14.1 ± 0.21
L. officinalis	Leaves: sedative (Piri, Personal information)	16.2 ± 0.59
M. officinalis	Leaves: anti-viral (Fintelmann & Weiss, 2004)	20.3 ± 0.19

The data are displayed with mean ± standard deviation of three replications. The contents of total phenolics in plants were expressed as gallic acid equivalent (GAE) per 1 g dry weight.

3.2. Determination of total flavonoids

The concentrations of total flavonoids of five Iranian medicinal plants varied between 0.22 and 10.0 mg of CE/g dw (Table 2). Leaves of *M. officinalis* exhibited the highest flavonoids concentration followed by leaves of *L. officinalis*, flowers of *S. lavandulifolium* and root of *V. officinalis*. The flowers of *A. kurdica* had the lowest flavonoids concentration.

3.3. Antioxidant activities

The total antioxidant activities quantified by VCEAC assay are presented in Table 2. ANOVA revealed significant differences between extracts (Fig. 1) with respect to total antioxidant activity ($F(4,10) = 113.15$; $p < 0.0001$). Fisher test did not reveal any significant difference between leaves of *M. officinalis* and of *L. officinalis* with respect to total antioxidant activity ($p > 0.05$).

Flowers of *S. lavandulifolium*, root of *V. officinalis* and flowers of *A. kurdica* exhibited significantly less antioxidant activity than Leaves of *M. officinalis* and leaves of *L. officinalis* ($p < 0.01$; $p < 0.001$; $p < 0.001$, respectively).

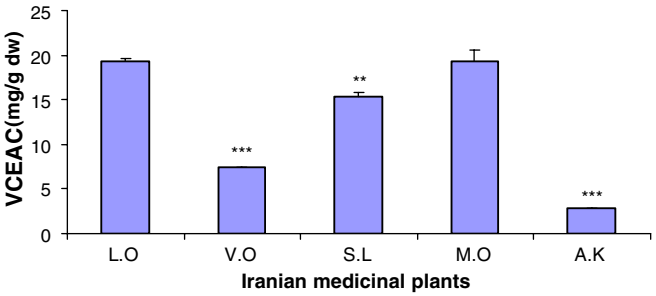

Fig. 1. Comparison of total antioxidant activities for parts of some Iranian medicinal plants estimated by VCEAC assay and expressed as vitamin C equivalent (VCE) per g dry weight. L.O, V.O, S.L, M.O, A.K stand for leaves of *L. officinalis*, root of *V. officinalis*, flowers of *S. lavandulifolium*, leaves of *M. officinalis* and flowers of *A. kurdica*, respectively. Data represent mean ± standard deviation of three replications. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

4. Discussion

The total phenolic and the total flavonoid contents of 1 g dry weight of plant parts traditionally used ranged from 2.15 to 20.3 mg of GAE and from 0.22 to 10.0 mg of CE, respectively (Tables 1 and 2). The total phenolics and the total flavonoids showed the similar tendency in ranking: leaves of *M. officinalis* followed by leaves of *L. officinalis*, root of *V. officinalis*, flowers of *S. lavandulifolium* and flowers of *A. kurdica*. The three first medicinal plant parts are potentially rich sources of natural antioxidants. However, flowers of *A. kurdica* are poor in polyphenols, their total phenolic and flavonoid amounts were approximately ninefold and 45-fold lower than leaves of *M. officinalis*, respectively. The medicinal plants have the same geographic origin and grow in the same natural conditions; nevertheless the plants belong to different families and the parts used in this study are not the same. It is well known that the amount of total phenolics vary in different parts of the same plant, moreover it has been reported that the amount of total phenolics vary with respect to families and varieties (Djeridane et al., 2006; Kahkonen et al., 1999; Kaur & Kapoor, 2002; Romani et al., 2003; Sellappan & Akoh, 2002). To evaluate antioxidant activities VCEAC test developed by Kim, Chun, et al. (2003) was employed. This test is a good method for measuring the antioxidant activity of extracts or individual chemical compounds (Chun, Kim, Moon, Kang, & Lee, 2003; Kim, Chun, et al., 2003; Kim, Jeong, & Lee, 2003). The different plant parts used display scavenging activities for ABTS radical. We found that the total antioxidant activities varied greatly among the different parts, since they ranged from 2.8 to 19.3 mg of VCE/g dw (Table 2). Based on this data, we can classify medicinal plants into two groups. The first one showing an identical and high antioxidant activity

Table 2
Total flavonoid contents in parts of some Iranian medicinal plants and their total antioxidant activities quantified by VCEAC assay

Scientific name	Total flavonoids (mg CE/g dw)	VCEAC (mg VCE/g dw)
A. kurdica	0.22 ± 0.004	2.8 ± 0.05
V. officinalis	1.1 ± 0.04	7.36 ± 0.09
S. lavandulifolium	4.02 ± 0.02	15.4 ± 0.47
L. officinalis	6.18 ± 0.18	19.2 ± 0.42
M. officinalis	10.0 ± 0.32	19.3 ± 1.35

The data are displayed with mean ± standard deviation of three replications. The contents of total flavonoids in plant parts and their total antioxidant activities estimated by VCEAC assay were expressed as catechin equivalent (CE) and vitamin C equivalent (VCE) per 1 g dry weight, respectively.

profile constituted by leaves of *L. officinalis* and of *M. officinalis*. The second one exhibiting low antioxidant activity constituted by root of *V. officinalis*, flowers of *S. lavandulifolium* and flowers of *A. kurdica*. The difference between these two groups was significant with respect to antioxidant activity (Fig. 1). Antioxidants are substances that delay the oxidation process, inhibiting the polymerization chain initiated by free radicals and other subsequent oxidizing reactions (Halliwell & Aruoma, 1991). Phenolic constituents, such as flavonoids, phenolic acids and tannins are well known for their high antioxidant activity (Rice-Evans, Miller, & Paganda, 1996; Shahidi, Janitha, & Wanasundara, 1992). Epidemiological studies suggest that the consumption of flavonoid-rich foods protects against human diseases associated with oxidative stress, like coronary heart disease and cancer (Duthie, Duthie, & Kyle, 2000; Lambert & Yang, 2003; Renaud & de Lorgeril, 1992). The protective effect provided by fruits and vegetables against cancer, cardio and cerebrovascular diseases, has been attributed to their antioxidant compounds (Ames, 1983; Gey, 1990). The majority antioxidant capacity of plants is not only represented by vitamin C, vitamin E or β-carotene, but is also due to other compounds such as polyphenols which have a strong antioxidant potential (Bors & Saran, 1987). Many studies indicate a linear relationship between total phenolics and antioxidant activity (Djeridane et al., 2006; Kim, Chun, et al., 2003; Kim, Jeong, et al., 2003). In this study, we found that phenolic compounds are major contributors to antioxidant activity, since total phenolics and antioxidant activity showed a good correlation with a correlation coefficient of $R^2 = 0.961$ (Fig. 2). However, antioxidant capacity and total flavonoids showed a relatively weak relationship with a correlation coefficient of $R^2 = 0.817$ (Fig. 3). A similar result was previously observed by Kim, Chun, et al. (2003). Our results are in agreement with previous reports that the phenolic compounds contribute significantly to the antioxidant activity in medicinal plants (Cai, Luo, Sun, & Corke, 2004; Djeridane et al., 2006; Tang et al., 2004; Wong et al., 2006).

In conclusion, the amount of phenolics, flavonoids and related total antioxidant activity of some Iranian medicinal plant parts were evaluated. Antioxidant activity varied greatly among the different plant parts used in this study, but it was highly correlated with the content of polyphenolics. Therefore, we take an interest in leaves of *M. officinalis* and of *L. officinalis*, since they exhibited important antioxidant activities and present a good source of natural antioxidants. After this comparative study, our objective will be identification and determination of the amount of individual polyphenolics responsible for the majority of antioxidant activity in leaves of these two plants.

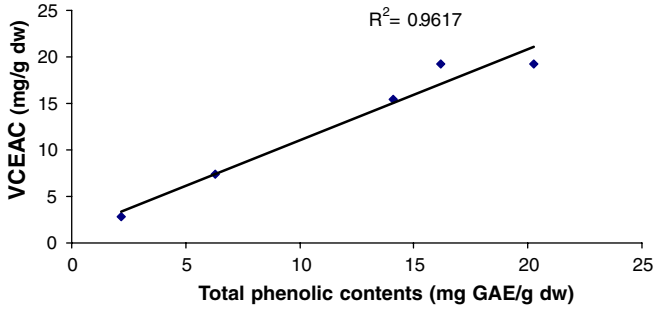

Fig. 2. Positive correlation between total phenolics and VCEAC for some Iranian medicinal plants.

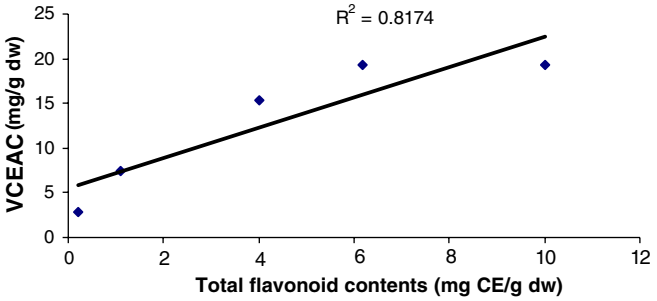

Fig. 3. Positive correlation between total flavonoids and VCEAC for some Iranian medicinal plants.

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