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Antioxidant capacities and total phenolic contents of infusions from 223 medicinal plants

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ABSTRACT

In order to find new sources of natural antioxidants, antioxidant capacities of infusions from 223 medicinal plants were systemically evaluated using ferric-reducing antioxidant power (FRAP) and Trolox equivalent antioxidant capacity (TEAC) assays, and their total phenolic contents (TPC) were measured by Folin–Ciocalteu method. A very weak correlation (R^2 = 0.1563) between the TEAC value and the FRAP value suggested that the components capable of scavenging free radicals could be different from those reducing oxidants in these plants. A significant correlation (R^2 = 0.7549) between TEAC value and TPC as well as a very weak correlation (R^2 = 0.1966) between FRAP value and TPC implied that phenolic compounds in these plants could be the main components contributing to scavenging free radical activities, but not be responsible for reducing oxidant abilities. Several plants possessing the high antioxidant capacities and TPC have been screened out, which could be potential rich resources of natural antioxidants, and could be developed into herbal infusion, functional food or pharmaceuticals for prevention and treatment of diseases caused by oxidative stress.

1. Introduction

The oxidative damages caused by reactive oxygen species on lipids, proteins and nucleic acids may trigger various chronic diseases. Several studies have shown that reactive oxygen species are involved in the etiology of many diseases, such as aging, cancer, atherosclerosis, coronary heart diseases, diabetes, asthma and rhinitis (Barnham et al., 2004; Eberhardt et al., 2000; Finkel and Holbrook, 2000). The body's non-enzymatic antioxidant defense system is made up of some antioxidants, such as vitamin C, vitamin E, vitamin K and glutathione. The exogenous antioxidants are mainly comprised of synthetic and natural antioxidants. However, there is widespread agreement that some synthetic antioxidants such as butylhydroxyanisole and butylhydroxytoluene need to be replaced with natural antioxidants because they were found to be toxic and carcinogenic in animal models (Botterweck et al., 2000; Ito et al., 1986; Safer and Al-Nughamish, 1999). Therefore, it is very important to find out new sources of safe and inexpensive antioxidants of natural origin.

Fruit, vegetable, cereal, edible macro-fungi and microalgae are known to be rich sources of natural antioxidants (Deng et al., 2012a,b; Fu et al., 2011; Guo et al., 2012; La Vecchia et al., 2001; Lako et al., 2007; Li et al., 2007). The best health and nutrition results can be achieved not only from the consumption of fruits and vegetables with high antioxidant capacities, but also from medicinal herbs and plants. Medicinal plants are another important source for a wide variety of natural antioxidants. Several studies indicated that some medicinal plants possess more potent antioxidant activity than common fruits and vegetables (Cai et al., 2004; Dragland et al., 2003). Medicinal plants have been used to treat human diseases for thousands of years. People are becoming increasingly interested in medicinal plants because of their good therapeutic performance and low toxicity. Since traditional medicinal plants and food are believed to share a common origin in Chinese tradition, it is very difficult to distinguish traditional medicinal plants from food. In fact, many medicinal plants have been used as flavors, pigments, and foods. The medicinal plants are thought to be a rich and natural source of functional food and pharmaceuticals. The health benefits of medicinal plants are thought to arise partly from potential effects of their antioxidants on the reactive oxygen species produced in the human body. In addition, several studies have indicated that medicinal plants possess more potent antioxidant activity than common dietary plants, and phenolic compounds were a major contributor to the antioxidant activity of these plants (Cai et al., 2004; Dragland et al., 2003). In recent years, studies on antioxidant activities of medicinal plants have increased remarkably due to increased interest in their potential of being used as a rich and natural source of antioxidants (Boulanouar et al., 2013; Cai et al., 2004; Goncalves et al., 2013; Inayatullah et al., 2012; Jaberian

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et al., 2013; Li et al., 2008a; Lizcano et al., 2010; Silva et al., 2007; Tlili et al., 2013; Zhang et al., 2011).

The antioxidant activities of the plant extracts largely depend on the composition of the extracts. Sample preparation is the crucial first step in the study of antioxidant property of plant, because it is necessary to extract antioxidants from the plant material for evaluation of antioxidant activity as well as further separation and identification of antioxidants. Several methods have been developed for the extraction of antioxidants from plant, and the solvent extraction using water, organic solvent, or the combination of them under the different ratios are widely used. Aqueous methanol between 50% and 80% is usually used for the extraction of antioxidant from medicinal plants (Cai et al., 2004; Li et al., 2008a). However, the use of large amount of organic solvents poses health and safety risks to researchers, and is environmentally unfriendly. In addition, methanol is inappropriately used in food and pharmaceutical industries because it is toxic. On the other hand, medicinal plants are traditionally boiled in water, and the extracts are used for consumption. The boiling water method eliminates the use of organic solvents. The boiling water extracts (herbal infusions) of medicinal plants have been consumed for several thousands of years in China, and they proved to be non-toxic. If the extraction solvents or extraction methods are changed, some harmful substances in medicinal plants could be extracted, and safety of the extracts need be evaluated renewedly. Because water has much lower toxicity than methanol, boiling water extraction method could be a better choice to obtain antioxidant-rich extracts from plants. When the extract possessing strong antioxidant activity is nontoxic, further isolation and purification of antioxidant components is not necessary because health benefits of the extract might be from additive and synergistic effects of phytochemicals in the extract (Liu, 2003). At this condition, the extract can be directly used as components of functional food or complex traditional medicines.

In this study, antioxidant capacities and total phenolic contents of infusions from 223 medicinal plants used most commonly in China were evaluated, and several plants possessing the high antioxidant capacities and total phenolic contents have been screened out, which could be potential rich resources of natural antioxidants, and could be developed into herbal tea, functional food or pharmaceuticals for prevention and treatment of diseases caused by oxidative stress.

2. Experimental

2.1. Chemicals and plant materials

Iron(III) chloride 6-hydrate, iron(II) sulfate 7-hydrate, acetic acid, hydrochloric acid, acetic acid, sodium acetate, potassium persulphate and sodium carbonate were obtained from Tianjin Chemical Factory (Tianjin, China). 2,2′-Azinobis(3-ethylbenothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), Folin–Ciocalteu's reagent and gallic acid were purchased from Sigma Aldrich (St. Louis, MO, USA). All chemicals used in the experiment were of analytical grade. All medicinal plants used in this study were purchased from Beijing Tong-Ren-Tang drug retail outlet in Guangzhou, China.

2.2. Sample preparation

The dry plant samples were ground to a fine powder with a special grinder for herbal medicine, and 0.5 g of the powder was extracted with 10 mL of deionized water at 100 °C for 30 min in a water bath. The mixture was then cooled to room temperature and centrifuged at 3500 × *g* for 10 min. The supernatant was recovered for the evaluation of antioxidant capacity and total phenolic content.

2.3. Ferric-reducing antioxidant power (FRAP) assay

The FRAP assay was carried out according to the literature (Benzie and Strain, 1996). Briefly, the FRAP reagent was prepared from sodium acetate buffer (300 mM, pH 3.6), 10 mM TPTZ solution (40 mM HCl as solvent) and 20 mM iron (III) chloride solution in a volume ratio of 10:1:1, respectively. The FRAP reagent was prepared fresh daily and warmed to 37 °C in a water bath before use. One hundred microliters of the diluted sample was added to 3 mL of the FRAP reagent. The absorbance of the reaction mixture was then detected at 593 nm after 4 min in room temperature. The standard curve was constructed using $FeSO_4$ solution, and the results were expressed as μmol Fe(II)/g dry weight of plant material.

2.4. Trolox equivalent antioxidant capacity (TEAC) assay

The TEAC assay was carried out to determine the free radical scavenging capacity using the $ABTS^{\bullet+}$ radical cation, according to the literature (Re et al., 1999). The $ABTS^{\bullet+}$ stock solution was prepared from 7 mM $ABTS^{\bullet+}$ and 2.45 mM potassium persulfate in a volume ratio of 1:1, and then should be incubated in the dark for 16 h at room temperature and used within 2 days. The $ABTS^{\bullet+}$ working solution was prepared by dilution of the stock solution with ethanol to an absorbance of 0.70 ± 0.05 at 734 nm. All samples were diluted approximately to provide 20–80% inhibition of the blank absorbance. One hundred microliters of the diluted sample was mixed with 3.8 mL $ABTS^{\bullet+}$ working solution. The absorbance of the reaction mixture was then detected at 734 nm after 6 min of incubation at room temperature, and the percent of inhibition of absorbance at 734 nm was calculated. Trolox solution was used as a reference standard, and the results were expressed as μmol Trolox/g dry weight of plant material.

2.5. Determination of total phenolic content

The total phenolic contents were determined using Folin–Ciocalteu method according to the literature (Singleton and Rossi, 1965). Briefly, diluted sample (0.50 mL) was added to 1:10 diluted Folin–Ciocalteu reagent (2.5 mL). After 4 min, saturated sodium carbonate solution (about 75 g/L, 2 mL) was added. After 2 h of incubation at room temperature, the absorbance of the reaction mixture was measured at 760 nm. Gallic acid was used as a reference standard, and the results were expressed as milligram gallic acid equivalent (mg GAE)/g dry weight of plant material.

All the experiments were performed in triplicate, and the results were expressed as mean ± SD (standard deviation). The correlation between the antioxidant capacities and total phenolic contents was analyzed using the simple linear regression, and the correlation coefficient (R^2) was calculated.

3. Results and discussion

3.1. Antioxidant capacities of the selected medicinal plants

The antioxidant capacity of the plant extract depends on, not only the composition of the extract but also the test system. It can be influenced by a large number of factors, and cannot be fully evaluated by one single method. A reliable antioxidant protocol requires the measurement of more than one property relevant to either the extract or biological system because most natural antioxidants and