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The influence of different treatments on the free radical scavenging activity of burdock and variations of its active components

Fu-An Chen [a,*], An-Bang Wu [b], Chau-Yang Chen [a]

[a] Tajen Institute of Technology, No. 20, Wei-Shin Road, Yan-Pu Shiang, Pingtung 907, Taiwan, ROC
[b] Graduate Institute of Pharmaceutical Sciences, Taipei Medical University, No. 250 Wu-Hsing Street, Taipei 110, Taiwan, ROC

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Abstract

The root of burdock (*Arctium lappa* L.) has long been cultivated as a popular vegetable in Taiwan and Japan for dietary use and folk medicine. The present study investigated the influence of the different treatments of peeling and heat treatment on (1) the free radical scavenging activity of burdock, using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and on (2) variations of its active components, chlorogenic acid and caffeic acid, by a HPLC method. Treatments were divided into four groups: group I, root of burdock without heat treatment; group II, peeled root of burdock without heat treatment; group III, root of burdock with heat treatment; and group IV, peeled root of burdock with heat treatment. Freeze-dried powders from both the root and peeled root of burdock, after heat treatment, had poor physical properties due to the apparent coagulation according to visual observations. The active phenolic components, chlorogenic acid and caffeic acid, existed mainly in the skin of burdock root, and the content of chlorogenic acid was much higher than that of caffeic acid. Burdock possessed significant free radical scavenging activity, which was mainly attributed to chlorogenic acid, whose free radical scavenging activity was similar to that of caffeic acid and higher than that of vitamin E. Peeling of the root greatly decreased the free radical scavenging activity and the concentrations of these two active components, due to elimination of the components in the discarded skin. Heat treatment slightly decreased the free radical scavenging activity, which was partially due to the degradation of chlorogenic acid.

Keywords: *Arctium lappa*; Burdock; Free radical scavenging activity; 1,1-Diphenyl-2-picryl-hydrazyl; HPLC

1. Introduction

Free radicals, formed by various environmental chemicals as well as endogenous metabolism, cause oxidative damage to DNA, lipids and proteins, resulting in failure of cellular functions through which a number of conditions such as tumors, inflammation, shock, atherosclerosis, diabetes, and ischemia can occur (Jin & Chen, 1998; Kasai, Fukada, Yamaizumi, Sugie, & Mori, 2000; Wallace, 1997). Consumption of dietary antioxidants from plant materials has been associated with a lowered incidence of diseases due to the oxidative stress from free radicals (Ames, 1983; Leong & Shui, 2002); accordingly, dietary antioxidants have recently garnered increased research interest.

Burdock (*Arctium lappa* L.) was introduced from Japan into Taiwan about 70 years ago and has long been cultivated as a vegetable in Taiwan for dietary use (Han, 1995). Burdock is also used as a folk medicine, as a diuretic and antipyretic (Kan, 1981). It has become a popular health drink in Taiwan in the past decade. Several studies have reported that the root of burdock possesses various pharmaceutical activities including antibacterial activity (Chow, Wang, & Duh, 1997), desmutagenic activity (Morita, Nishijima, & Kada, 1984), antioxidant activity (Duh, 1998; Lin, Lin, Yang, Chuang, & Ujiie, 1996), hepatoprotective efficacy (Lin et al., 2000, 2002), and anti-inflammatory activity

* Corresponding author.
E-mail address: fachen@ccsun.tajen.edu.tw (F.-A. Chen).

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(Lin et al., 1996), among which the hepatoprotective efficacy, anti-inflammatory activity, and antioxidant activity are associated with free radical scavenging activity. Therefore, free radical scavenging activity is an important biological activity of burdock.

Hot water is frequently used as a solvent for household cooking and for preparing extracts of burdock for pharmacological study (Duh, 1998; Lin et al., 2000, 2002; Lin et al., 1996). In addition, peeling of burdock is the usual process before it is cooked in the household and prepared as a health drink. Although the pharmacological activity has been intensively investigated, very little research concerning the effect of different treatments on its pharmacological activity and variations of its active components has been carried out. The present study estimates the influence of peeling and heat treatment on the free radical scavenging activity of burdock using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and on variations of its active components, chlorogenic acid and caffeic acid, by an HPLC method.

2. Materials and methods

2.1. Materials

The root of burdock (*Arctium lappa* L.) was kindly provided by Gueilai Community Developmental Institute in Pingtung County, southern Taiwan. Chlorogenic acid, caffeic acid, vitamin E (±-α-tocopherol), and DPPH (2,2-diphenyl-1-picrylhydrazyl) were purchased form Sigma Chemicals (St. Louis, MO, USA). LC grade methanol was from Fisher Chemicals (Springfield, NJ, USA), and glacial acetic acid was from Merck (Darmstadt, Germany). The Cosmosil 5C18-MS-II reverse-phase HPLC column (250×4.6 mm i.d.) was the product of Nakalai Tesque (Kyoto, Japan). All other chemicals were of analytical reagent grade.

2.2. Preparation of freeze-dried powders by different treatments

The root and peeled root of burdock were juiced and filtered to obtained crude juices, which were classed as groups I and II, respectively. Groups III and IV were obtained from crude juices of groups I and II, respectively, after heat treatment at 98 ± 0.5 °C in a water bath (Frago Instrument, Taipei, Taiwan) for 10 min. Each crude juice was further lyophilized to obtain freeze-dried powders of groups I to IV for the following study. Parts of the obtained freeze-dried powders were analysed immediately to estimate their free radical scavenging activities and active compounds, chlorogenic acid and caffeic acid. Residual freeze-dried powders were stored in an electronic dry cabinet (Komry, Taiwan) and were visually inspected each month to assess their physical stability.

2.3. DPPH free radical scavenging assay

The DPPH free radical is a stable free radical, which has been widely used as tool to estimate free radical scavenging activities of antioxidants (Jin & Chen, 1998). The free radical scavenging activity, of burdock with different treatments, on the DPPH radical was conducted using the method described by Parejo, Codina, Petrakis, and Kefalas (2000). A 6×10^{-5} M solution of DPPH in methanol was prepared. An aliquot (0.1 ml) of a methanol solution containing different concentrations of each burdock freeze-dried powder or authentic antioxidant was added to 3.9 ml of this solution. The decrease in absorbance at 515 nm was measured at each predetermined checkpoint. The rate of DPPH disappearance was calculated by fitting each curve of plotted values of DPPH remaining (%) against time to a logistic equation.

2.4. HPLC assay conditions

A Shimadzu LC-10AT HPLC pump system equipped with an SPD-10A UV/VIS detector, C-R6A integrator, and Jasco 851-AS autosampler was used to analyze the active components, chlorogenic acid and caffeic acid, in freeze-dried powders with different treatments on a Cosmosil $5C_{18}$-MS-II column (i.d. 4.6×250 mm) at 320 nm. A mixture of MeOH/H_2O/HOAc (30: 69: 1, v/v) was used as the mobile phase at a flow rate of 1 ml/min and an injection volume of 20 μl.

2.5. Heat treatment of active compounds, chlorogenic acid and caffeic acid

A 5 ppm aqueous solution of chlorogenic acid and caffeic acid was prepared for the purposes of studying the effect of heat treatment on degradation of chlorogenic acid and caffeic acid. The solutions were incubated at 98 ± 0.5 °C in a water bath. An aliquot of the solution (500-μl) was removed at each pre-determined checkpoint and was assayed with HPLC assay method.

3. Results and discussion

3.1. Observations of the appearance of freeze-dried powders with different treatments

Each freeze-dried powder with different treatments was visually inspected to estimate its physical stability each month. As shown in Fig. 1, the appearance of freeze-dried powder, from either the root or peeled root

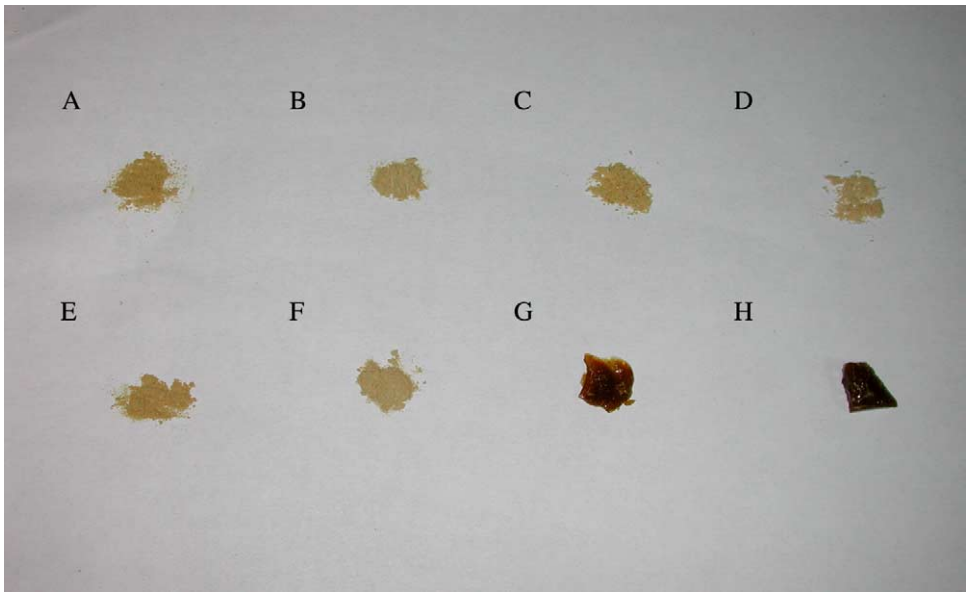

Fig. 1. Variations in the appearance of freeze-dried powders from the original (a: group I; b: group II; c: group III; d: group IV) and after 1 month (e: group I; f: group II; g: group III; h: group IV).

of burdock, without heat treatment, revealed no significant changes by visual observations after 1 month (Figs. 1(a), (b), (e) and (f)) or even until 6 months (data not shown), while that of root or peeled root of burdock with heat treatment had apparently coagulated according to visual observations after 1 month (Figs. 1(c), (d), (g) and (h)). The results indicate that freeze-dried powders of root and peeled root of burdock with heat treatment had poor physical stability. It was reported that burdock has abundant carbohydrates (Han, 1995). Freeze-dried powders from the root or peeled root of burdock with heat treatment apparently coagulated and were viscous, which may have resulted from changes in the carbohydrate composition during heat treatment.

3.2. DPPH free radical scavenging assay

In order to determine the influence of different treatments on free radical scavenging activity, a DPPH free radical scavenging assay was carried out. The disappearance rate of DPPH free radicals was used to express the free radical scavenging activity of each burdock freeze-dried powder. As shown in Fig. 2, all burdock freeze-dried powders possessed significant free radical scavenging activity in a dose-dependent manner. By comparisons between, either groups I and II, or groups III and IV, peeling greatly decreased the free radical scavenging activity of burdock, indicating that its active components mainly exist in the skin and that they predominate in its free radical scavenging activity. A slight decrease was found with heat treatment by comparison between, either groups I and III, or groups II and IV, implying that the slight decrease in free radical scavenging activity may have resulted from degradation of the active components. Phenolic compounds with antioxidant activity exist ubiquitously in plant materials, including herbs, fruits, and vegetables. As burdock has been reported to contain phenolic compounds, such as chlorogenic acid and caffeic acid (Chiu & Chang, 1992), its free radical scavenging activity was further estimated by DPPH free radical scavenging assay. As shown in Fig. 3, the rate of disappearance of the DPPH free radical increased dose-dependently with chlorogenic acid and caffeic acid indicating that these two phenolic components possess free radical scavenging activity. Compared with the authentic antioxidant vitamin E, the free radical scavenging activity decreased in the order chlorogenic acid $\fallingdotseq$ caffeic acid $>$ vitamin E. Results indicate that chlorogenic acid and caffeic acid possessed high free radical scavenging activity and that

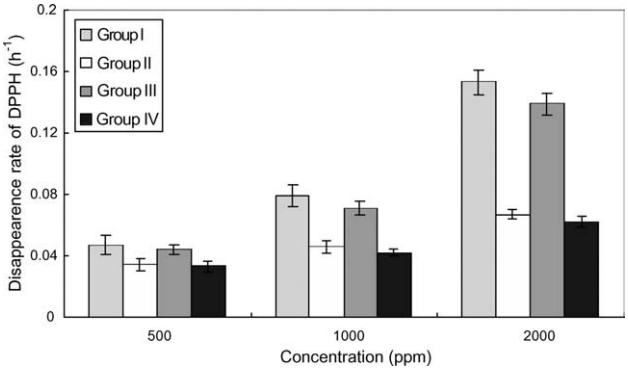

Fig. 2. Variations in free radical scavenging activity after different treatments.

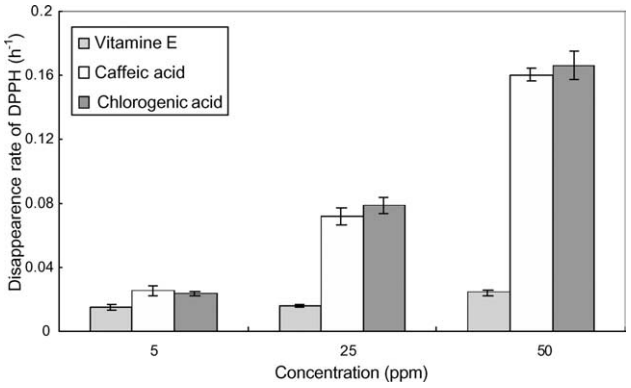

Fig. 3. The free radical scavenging activities of chlorogenic acid, caffeic acid, and vitamin E.

these two phenolic compounds had similar free radical scavenging activity efficacies.

3.3. HPLC assay for measuring the compounds in burdock freeze-dried powders with free radical scavenging activities

In order to determine the influence of different treatments on variations in components with free radical scavenging activities, an HPLC method was developed to quantify chlorogenic acid and caffeic acid in each group. The HPLC chromatograms of chlorogenic acid and caffeic acid standards are shown in Fig. 4(a). The retention times of chlorogenic acid and caffeic acid were about 6.0 and 8.8 min, with detection limits of 25 and 8 ng/ml ($S/N > 3$), respectively. The calibration curve was constructed by plotting chlorogenic acid and caffeic acid response areas vs. concentration which ranged from 0.25 to 4 μg/ml and 62.5 ng/ml–1 μg/ml, respectively. The correlation coefficient of the linear regression analysis was higher than 0.999. The intra-day and inter-day relative standard deviations (RSDs) of three replicate determinations for three consecutive days, at high, medium, and low concentrations of the calibration curve for chlorogenic acid and caffeic acid, were all below 6%. On the basis of the above results, the developed HPLC method was judged to be reliable and applicable for quantifying variations in these active components after different treatments. As shown in Figs. 4(b)–(e), both chlorogenic acid and caffeic acid existed in each group, but the peak of chlogenic acid was much higher than that of caffeic acid. Contents of chlorogenic acid and caffeic acid in each group were calculated and are shown

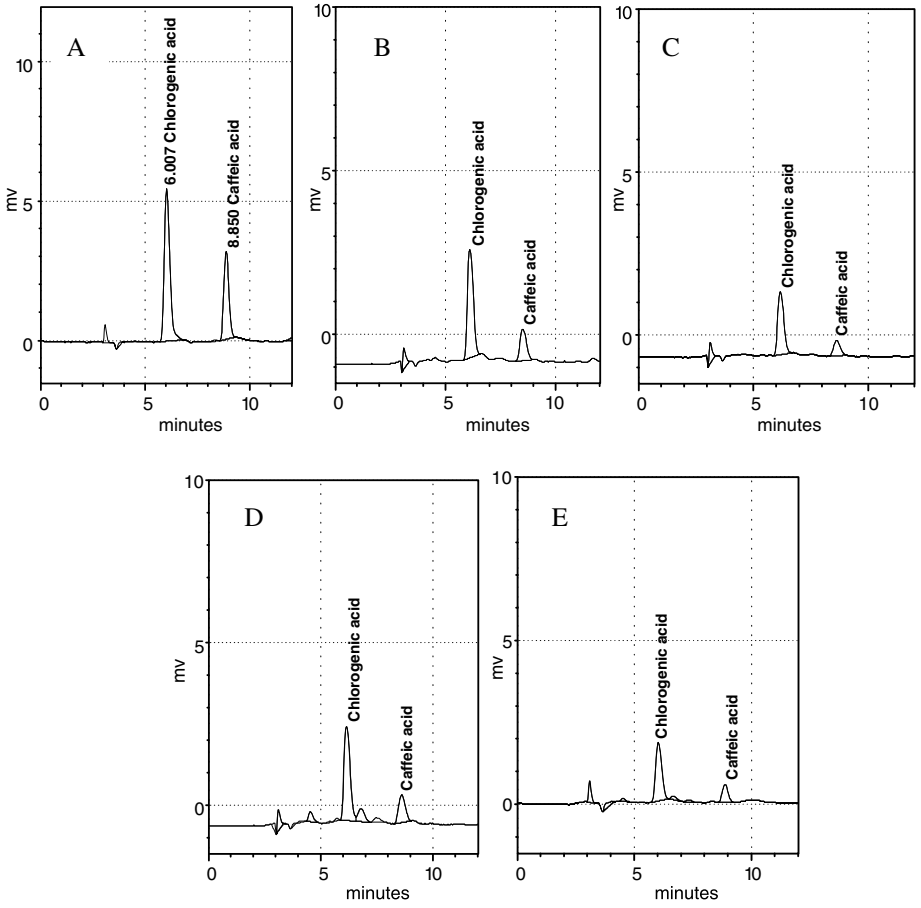

Fig. 4. HPLC chromatograms of chlorogenic acid and caffeic acid standards (a), group I (b), group II (c), group III (d), and group IV (e).

in Fig. 5. The content of chlorogenic acid in the four groups was about 15-fold higher than that of caffeic acid, indicating that chlorogenic acid is the major phenolic component with free radical scavenging activity in burdock. By subtracting the contents of these two components of group I from those of group II, we can estimate that about 40% of these two components existed in the skin of the burdock root. The weight of the skin of the whole root of burdock accounted for only about 10%, but the skin contained about 40% of these two components. The results indicate that these two components mainly exist in the skin of the burdock root, and that peeling treatment greatly decreased the contents of these two components. By comparison between, either groups I and II, or groups III and IV, the enormous decrease in contents of these two components, after peeling treatment, was attributed to the elimination of most of these two components in the skin of burdock root, which resulted in dramatic decreases in the free radical scavenging activity. Moreover, heat treatment caused slight decreases in the content of chlorogenic acid but not caffeic acid, according to a comparison between either groups I and III or groups II and IV. The results imply that the slight decrease could be attributed to degradation of chlorogenic acid, based on other peaks from degradation products found in the HPLC chromatograms of groups III and IV (Figs. 4(d) and (e)). Therefore, the chemical stability of these two phenolic compounds after heat treatment was further studied. As shown in Fig. 6, only the content of chlorogenic acid significantly decreased after heat treatment in a time-dependent manner. The peaks from degradation products found in the HPLC chromatogram of chlorogenic acid after heat treatment (data not shown) agree with those of groups III and IV. The results indicate that the slight decrease in content of chlorogenic acid is due to its degradation after heat treatment and is related to the slight decrease in free radical scavenging activity. The overall results for variations of the contents of these two components, after different treatments, were in agreement with those of the DPPH free radical scavenging assay and indicated that chlorogenic acid and caffeic acid are components with free radical scavenging activities, dominated by the high content of chlorogenic acid in the root.

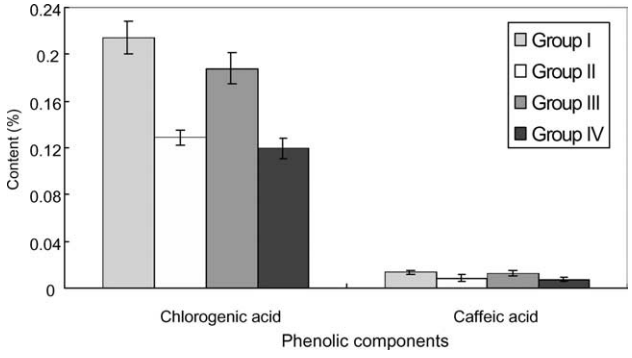

Fig. 5. Variations in the contents of chlorogenic acid and caffeic acid in burdock after different treatments.

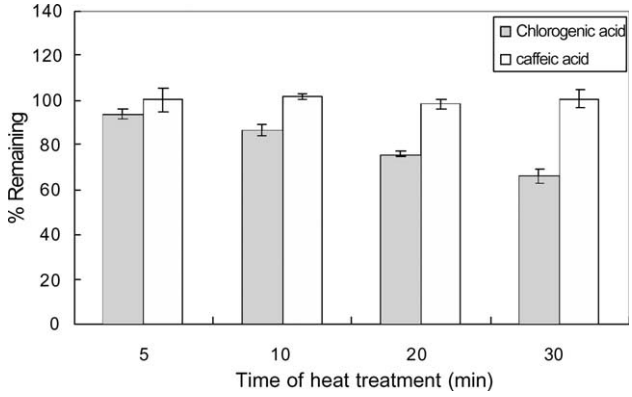

Fig. 6. Variations in the contents of chlorogenic acid and caffeic acid during heat treatment.

In conclusion, the active phenolic components, chlorogenic acid and caffeic acid, exist mainly in the skin of burdock root, and the content of chlorogenic acid was much higher than that of caffeic acid. Burdock possesses significant free radical scavenging activity, which was mainly attributed to chlorogenic acid, whose free radical scavenging activity is similar to that of caffeic acid and higher than that of vitamin E. Peeling greatly decreased these two active components of burdock and its free radical scavenging activity, which resulted from elimination of most of these two components in the skin of the root. Heat treatment slightly decreased the free radical scavenging activity, which was partially due to the degradation of chlorogenic acid. From a practical point of view, the unpeeled root of burdock without heat treatment is suitable for preparation of drinks or other health products because it possesses higher free radical scavenging activity, higher contents of active components, and greater stability, including physical and chemical stability.

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