Antioxidant Activity of Burdock (*Arctium lappa* Linné): Its Scavenging Effect on Free-Radical and Active Oxygen

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ABSTRACT: The antioxidant activity and free-radical and active oxygen-scavenging activity of burdock extracts were investigated. Of the solvents used for extraction, water yielded the greatest amount of extract that exhibited the strongest antioxidant activity. Water extracts of burdock (WEB) and hot water extracts of burdock (HWEB) exhibited comparable and marked activity on inhibition of linoleic acid peroxidation, indicating that heat treatment did not alter the antioxidant activity of WEB. WEB and HWEB produced significantly lower ($P < 0.05$) malondialdehyde (MDA) in both linoleic acid and liposome model systems than did the control. Moreover, mixtures of tocopherol (Toc), WEB, and HWEB exhibited a remarkable synergistic antioxidant effect in a liposome system; WEB and HWEB thus potentiated the action of Toc. Furthermore, WEB and HWEB displayed a marked inhibitory effect on lipid peroxidation of rat liver homogenate *in vitro*. WEB and HWEB exhibited an 80% scavenging effect on α,α-diphenyl-β-picrylhydrazyl radical and marked reducing power, indicating that WEB and HWEB act as primary antioxidants. Both extracts at a dose of 1.0 mg exhibited a 60.4–65.0% scavenging effect on superoxide and an 80.5% scavenging effect on hydrogen peroxide. They also showed a marked scavenging effect on the hydroxyl radical. These results revealed that WEB and HWEB are also active as oxygen scavengers and as secondary antioxidants. Based on these results, termination of free-radical reactions and quenching of reactive oxygen species in burdock extracts are suggested to be, in part, responsible for the antioxidant activity of burdock extracts.
JAOCS 75, 455–461 (1998).

KEY WORDS: Active oxygen, antioxidant activity, burdock, free radical, hot water extracts of burdock, lipid peroxidation, reducing power, scavenging effect, synergistic effect, water extracts of burdock.

Lipid peroxidation not only lowers the nutritive value of food and deteriorates the flavor and taste but also causes aging, heart disease, stroke, emphysema, mutagenesis, and carcinogenesis (1). It is necessary therefore to suppress lipid peroxidation in food, not only to preserve the nutritive and aromatic qualities but also to maintain the food's safety and wholesomeness. Oxidation or deterioration of food can be prevented in several ways; for instance, addition of antioxidants to foods is the most effective means of retarding fat oxidation. Synthetic antioxidants, including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG), are widely used in the food industry, but their value has been questioned (2). Natural antioxidants, including tocopherol (Toc) and ascorbic acid, are less effective than synthetic antioxidants. Hence, there is a need to identify new natural antioxidants for use as safe and effective additives in the food industry (3).

Burdock (*Arctium lappa* Linné), a vegetable, is consumed and has been used daily in beverages in China for centuries. Analysis of its components and its desmutagenic effect have been reported (4,5). Few reports, however, have addressed the antioxidant activity of burdock. Solvent extraction is the major method used to isolate natural antioxidants. Methanol is frequently used, but water remains the preferred solvent for purposes of food hygiene. Whether water extracts of burdock exhibit antioxidant activity, however, remains unclear. Burdock beverages are prepared from hot water extracts. It is necessary therefore to assay the antioxidant activity of water extracts of burdock (WEB) or hot water extracts of burdock (HWEB).

Antioxidants are classified into two groups, namely, primary or chain-breaking antioxidants and secondary antioxidants, depending on their mechanism of action. The former react with lipid peroxy radicals to convert them to stable products; this group includes chain breakers (or free radical inhibitors) and peroxide decomposers. Secondary antioxidants, such as oxygen scavengers, reduce the rate of chain initiation (6). Five antioxidative caffeoylquinic acid derivatives were isolated from methanol extracts of burdock (7), but the mechanism of action of burdock extracts remains unknown.

The present study was undertaken to examine the antioxidant activity of WEB and HWEB and to elucidate the mechanism of action of burdock extracts.

MATERIALS AND METHODS

Materials. Dried burdock (*A. lappa* Linné) roots were purchased from a local market in Pinton, Taiwan, Republic of China. Linoleic acid, NADH, and phenazine methosulfate (PMS) were purchased from E. Merck Co. (Darmstadt, Germany). Nitro blue tetrazolium (NBT), α,α-diphenyl-β-picrylhydrazyl (DPPH), and 5,5-dimethyl-pyrroline *N*-oxide

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(DMPO) were obtained from Sigma Chemical Co. (St. Louis, MO).

Extraction. Burdock (2.5 g) was extracted in 50 mL of *n*-hexane, chloroform, ethanol, methanol, or distilled water, in a shaker incubator at 25°C for 8 h. The extracts were filtered, and the residue was re-extracted under the same conditions. The combined filtrates were evaporated to dryness under vacuum and weighed to measure the yield of soluble constituents. The yields, averages of triplicate analyses, are listed in Table 1.

Preparation of WEB and HWEB. WEB and HWEB were prepared as described above, except for HWEB, burdock (20 g) was extracted in boiling distilled water (400 mL) for 5 min. The average yield of triplicate analyses of HWEB was 7.12 g.

Antioxidant activity in a linoleic acid system. An antioxidant activity assay was carried out by using the linoleic acid system (8). Extracts (0.2 mL) of each sample were added to a solution of linoleic acid (0.13 mL), 99.8% ethanol (10 mL), and 0.2 M phosphate buffer (pH 7.0, 10 mL). The total volume was adjusted to 25 mL with distilled water. The reaction mixture was incubated at 40°C, and the degree of oxidation was measured according to the thiocyanate method (9) by sequentially adding ethanol (10 mL, 75%), ammonium thiocyanate (0.2 mL, 30%), sample solution (0.2 mL), and ferrous chloride (20 mM in 3.5% HCl) solution (0.2 mL). After the mixture was stirred for 3 min, the peroxide value was determined by reading the absorbance at 500 nm, and the percentage inhibition of linoleic acid peroxidation, 100 − [(absorbance increase of sample/absorbance increase of control) × 100] was calculated to express antioxidant activity. All tests and analyses were run in triplicate and averaged.

Estimation of anti-$FeCl_2$-H_2O_2-stimulated linoleic acid peroxidation. The effect of anti-$FeCl_2$-H_2O_2-stimulated linoleic acid peroxidation was determined by the methods of Tamura and Shibamoto (10). Extracts (0.2 mL, 0–16.3 mg) were added to a solution of 0.1 M linoleic acid (0.2 mL), 2.0 mM $FeCl_2 \cdot 4H_2O$ (0.2 mL), 2.0 mM H_2O_2 (0.2 mL), and 0.2 M phosphate buffer (pH 7.4, 5.0 mL). The reaction mixture was incubated at 37°C for 24 h. After incubation, 0.2 mL BHA (20 mg/mL), 1.0 mL thiobarbituric acid (TBA) (1.0%), and 1.0 mL trichloroacetic acid (TCA) (10%) were added to the mixture, which was heated for 30 min in a boiling water bath. After cooling, 5.0 mL chloroform was added, and the mixture was centrifuged at 1000 × *g* to give a supernatant. Absorbance of the supernatant was measured spectrophotometrically at 532 nm. All analyses were run in triplicate and averaged.

Measurement of inhibition of malondialdehyde formation. Lecithin (300 mg) was sonicated in an ultrasonic cleaner (Branson 8210; Branson Ultrasonic Corporation, Danbury, CT) in 10 mM phosphate buffer (pH 7.4) for 2 h. The sonicated solution (10 mg liposome/mL), $FeCl_3$, ascorbic acid, and extracts (1.0 mg or 10 mg) were mixed to produce a final concentration of 3.08 mg liposome/mL, 123.2 μM $FeCl_3$, and 123.2 μM ascorbic acid. For assessment of synergistic action, α-Toc (2.0 μg) was added to the reaction mixture, then the desired amount of extract (1 or 10 mg) was added to the reaction mixture. Antioxidant action was measured after incubation for 1 h at 37°C by the method of Tamura and Shibamoto (10). The absorbance of the sample was read at 532 nm against a blank, which contained all reagents except lipid. The extinction coefficient of TBA-malondialdehyde product of 1.56×10^5 M^{-1} cm^{-1} was used to convert absorbance values into concentrations of secondary reaction products.

Estimation of anti-$FeCl_2$-ascorbic acid-stimulated lipid peroxidation in rat liver homogenate. Male Wister Imamichi rats, weighing 200 g, were used. Four grams of liver tissue were sliced and then homogenized with 22.5 mL of 150 mM KCl-Tris-HCl buffer (pH 7.2) and centrifuged at 5000 × *g* for 10 min to give a supernatant as liver homogenate.

The effect of anti-$FeCl_2$-ascorbic acid-stimulated lipid peroxidation was determined by the method of Yoden *et al*. (11). A mixture that contained 0.4 mL of liver homogenate, 0.1 mL of Tris-HCl buffer (pH 7.2), 0.2 mL of 6 mM ascorbic acid, 0.1 mL of 4 mM $FeCl_2$, and 0.2 mL of test compounds was incubated for 1 h at 37°C. After incubation, 0.5 mL of 0.1 N HCl, 0.5 mL of 0.9% sodium dodecyl sulfate (SDS), and 0.5 mL H_2O were added to the incubation solution. After shaking, 2 mL of 0.5% TBA was added. The mixture was heated for 30 min in a boiling water bath. After cooling, 5 mL *n*-butanol was added, and the mixture was shaken vigorously. The *n*-butanol layer was separated by centrifugation at 1000 × *g*. The absorbance of the sample was read at 532 nm against a blank, which contained all reagents except liver homogenate. All analyses were run in triplicate and averaged.

Reducing power. The reducing power of extracts was determined according to the method of Oyaizu (12). Extracts (0.326–1.63 mg) in phosphate buffer (2.5 mL, 2.0 M, pH 6.6) were added to potassium ferricyanide (2.5 mL, 1.0%), and the mixture was incubated at 50°C for 20 min. TCA (2.5 mL, 10%) was added to the mixture, which was then centrifuged at 650 × *g* for 10 min. The supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 0.1%), and the absorbance was read spectrophotometrically

TABLE 1
Yield and Antioxidant Activity of Burdock Extracted with Different Solvents

Solvent	Yield (g)[a]	Antioxidant activity (%)[b]
Water	1.63 ± 0.012[c]	96.3 ± 0.047[c]
Methanol	1.52 ± 0.014[d]	94.4 ± 0.205[d]
Ethanol	0.69 ± 0.047[e]	92.8 ± 0.368[d]
Chloroform	0.05 ± 0.001[f]	15.9 ± 1.429[e]
n-Hexane	0.03 ± 0.001[f]	0.0 ± 0.000[f]

[a]Based on 2.5 g of burdock for each solvent. Values are mean ± standard deviation of three replicate analyses.
[b]The antioxidant activity, determined by the thiocyanate method and the percentage inhibition of linoleic acid peroxidation, 100 − [(absorbance increase of sample/absorbance increase of control) × 100], was calculated to express antioxidant activity. Means within a column with different superscript letters (c–f) are significantly different ($P < 0.05$).

at 700 nm. Higher absorbance of the reaction mixture indicated greater reducing power.

Determination of effect on DPPH radical. The effects of WEB and HWEB on DPPH radical were estimated according to the method of Hatano *et al.* (13). Extracts were decolorized with cartridges (Sep-Pak C_{18}; Waters, Milford, MA) in distilled water (2.0 mL), and added to a methanolic solution (0.5 mL) of DPPH radical (the final concentration of DPPH was 0.2 mM). The mixture was shaken vigorously and left standing at room temperature for 30 min; absorbance of the resulting solution was then measured spectrophometrically at 517 nm. All tests and analyses were run in triplicate and averaged.

Scavenging of superoxide. The influence of extracts on generation of superoxide was evaluated by spectrophotometric measurement of the product on reduction of NBT (14). Superoxide was generated in a nonenzymic system. The reaction mixture, which contained extracts (0.16–3.2 mg) in distilled water, PMS (0.2 mL, 60 μM) in phosphate buffer (0.1 M, pH 7.4), 0.2 mL of NADH (677 μM) in phosphate buffer, and NBT (0.2 mL, 144 μM) in phosphate buffer, was incubated at ambient temperature for 5 min, and its absorbance was read spectrophotometrically at 560 nm against blank samples. All tests and analyses were run in replicate and averaged.

Scavenging of hydrogen peroxide. The ability of extracts to scavenge hydrogen peroxide was determined according to the method of Ruch *et al*. (15). A solution (2 mM) of hydrogen peroxide was prepared in phosphate-buffered saline (PBS, pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm by using the molar absorptivity of 81 M^{-1} cm^{-1}. Extracts (0.0163–1.0 mg) in distilled water were added to a hydrogen peroxide solution (0.6 mL). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution that contained extracts in PBS without hydrogen peroxide.

Hydroxyl radical scavenging activity. Hydroxyl radical reacts rapidly with the nitrone spin trap DMPO; the resultant DMPO-OH adduct can be detected by use of electron paramagnetic resonance (EPR) spectroscopy. The spectrum was recorded 2.5 min after extracts (1.0–81.5 mg, 0.5 mL) were mixed with H_2O_2 (10.0 mM, 0.2 mL), Fe^{2+} (10.0 mM, 0.2 mL), and DMPO (0.3 M, 0.2 mL) in phosphate buffer (pH 7.2) in an EPR spectrometer (Bruker ER 200D 10/12, Rheinstetten/Karlsruhe, Germany), set at the following conditions: receiver gain, 8×10^5; modulation amplitude, 1.0 G; scan time, 200 s; field, 3478.9 ± 50 G; time constant, 0.5 s (16).

Statistical analysis. Statistical analysis involved use of the Statistical Analysis System (17) software package. Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Duncan's Multiple Range tests.

RESULTS AND DISCUSSION

The yields and antioxidant activity of different solvents for burdock are listed in Table 1. The results indicated that the extract yield increased with increasing polarity of solvent. The extraction efficiency of solvents decreased in the order water > methanol > ethanol > chloroform > *n*-hexane. Of these five solvent extracts, water extracts exhibited the greatest yield and strongest antioxidant activity. The roots of burdock are edible and are commonly consumed, as are drinks derived from hot-water extracts of burdock. Therefore, we focused on the water extracts of burdock in the following study.

The antioxidant activity of different amounts of WEB and HWEB in a linoleic acid system with or without oxidation by $FeCl_2$-H_2O_2 is shown in Figure 1. Figure 1A shows that antioxidant activity of WEB and HWEB in linoleic acid oxidation increased with increasing amounts up to 1.63 mg, after which antioxidant activity remained nearly the same ($P > 0.05$) from 1.63 to 3.26 mg, indicating that the antioxidant activity of HWEB and WEB is thus closely related to the amount added. In general, no significant difference ($P > 0.05$) in inhibition of linoleic acid peroxidation was found between WEB and HWEB. This result reveals that heat treatment (100°C, 5 min) did not alter the antioxidant activity of burdock extract. This conclusion is meaningful to consumers because burdock drinks prepared from hot water extracts still retain a strong antioxidant effect. These properties make HWEB an excellent candidate for use as a natural antioxidant in processed food.

Iron salts are thought to react with H_2O_2, called the Fenton reaction, to make hydroxyl radicals, which bring about peroxidic reaction of lipids (18). The effect of WEB and HWEB on the formation of malondialdehyde (MDA) from linoleic acid oxidized by $FeCl_2$-H_2O_2 is shown in Figure 1B. The formation of MDA decreased with increasing amounts of WEB and HWEB. In the oxidation of linoleic acid, the formation of MDA was inhibited 67.5 and 68.2% in the presence of 8.15 mg of WEB and HWEB, respectively. In general, the

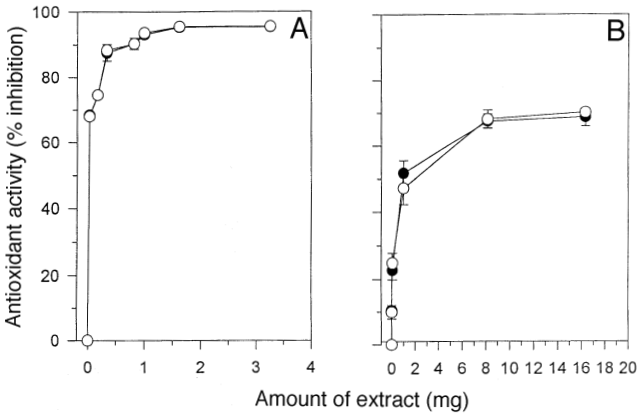

FIG. 1. Antioxidant activity of different amounts of water extracts of burdock (WEB) and hot water extracts of burdock (HWEB). (A) Antioxidant activity was determined by the thiocyanate method; (B) Antioxidant activity was measured by the thiobarbituric acid method, and the malondialdehyde formation from linoleic acid was oxidized by $FeCl_2$-H_2O_2. The percentage inhibition of linoleic acid peroxidation, 100 − [(absorbance increase of sample/absorbance increase of control)] × 100], was calculated to express antioxidant activity. ●, WEB; ○, HWEB.

antioxidant activities of WEB and HWEB in this model system were not significantly different ($P > 0.05$) (Fig. 1B). These results indicate that WEB and HWEB possess the activity for anti-lipid peroxidation.

To study in detail the burdock extracts in biological systems, the liposome model system was used for evaluation of antioxidant activity (19). Synergistic antioxidant activity of WEB and HWEB with Toc in the liposome system, as induced by $FeCl_3$ and ascorbic acid and measured by the TBA method, is plotted in Figure 2. A significant difference ($P < 0.05$) was found between the control and the liposome-containing HWEB and WEB (1.0 or 10.0 mg). The extracts at 1.0 mg show 2.85 and 2.67 nmole MDA/mg lipid for WEB and HWEB, respectively, and at 10 mg they show 0.60 nmole MDA/mg lipid for both WEB and HWEB. However, formation of MDA for the control is 3.68 nmole MDA/mg lipid, indicating that WEB at 1.0 and 10 mg inhibited 22.6 and 83.7% of peroxidation of lecithin, and HWEB at 1.0 and 10 mg inhibited 27.4 and 83.7% of peroxidation.

MDA is very reactive and takes part in cross-linking with DNA and proteins, and it also damages liver cells. (20). As seen in Figure 2, WEB and HWEB at 1.0 mg generated significantly ($P < 0.05$) less MDA than did 2.0 μg Toc, which failed to satisfactorily inhibit formation of MDA in the present study. It is evident that burdock extracts (WEB and HWEB) effectively inhibit formation of MDA. Furthermore, WEB and HWEB (1.0 or 10.0 mg) potentiated the antioxidant activity of Toc (2.0 μg). This finding implies that burdock extracts (WEB or HWEB) act synergistically with Toc. Toc, a well-known natural antioxidant, functions as a free-radical quencher in biological cells and localizes within the phospholipid bilayer of cell membranes to protect against biological lipid peroxidation (21). WEB and HWEB may also protect against damage to cell membranes because they reduce the level of lipid peroxides.

The inhibitory effect of different amounts of WEB and HWEB on MDA production in rat liver homogenate, induced by $FeCl_2$-ascorbic acid *in vitro,* is shown in Figure 3. The inhibition of MDA formation of rat liver homogenate in lipid peroxidation increased with increasing amounts of WEB and HWEB. In the peroxidation of rat liver homogenate, the formation of MDA was inhibited 96.5, 96.4, and 62.7% in the presence of 35 mg of WEB, HWEB, and Toc, respectively. The inhibitory effects of WEB and HWEB on the formation of MDA in rat liver homogenate were superior to that of Toc. In general, the antioxidant activities of WEB and HWEB on MDA production in rat liver homogenate were not significantly different ($P > 0.05$), but significant differences ($P < 0.05$) were found between 35 mg of Toc and 35 mg of either WEB or HWEB. These results indicate that WEB and HWEB displayed marked antioxidant activity in inhibiting peroxidation of rat liver homogenate *in vitro*.

It can be concluded from Figures 1, 2, and 3 that the extracts of burdock had strong antioxidative action not only in linoleic acid and liposome systems but also in a rat homogenate model system.

The antioxidant effect has been reported by some investigators to be concomitant with development of reducing power (22). The reducing power of WEB and HWEB increased with concentration (Fig. 4). Moreover, antioxidant activity correlated well with the reducing power of WEB ($r^2 = 0.90$) and HWEB ($r^2 = 0.88$). WEB and HWEB at 1.0 mg showed greater reducing power than did 0.01 mg of ascorbic acid

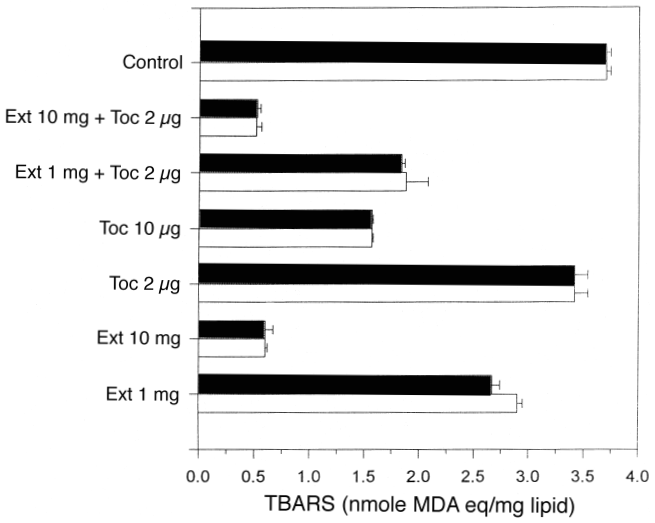

FIG. 2. Synergistic antioxidant activity of WEB and HWEB with α-tocopherol (Toc) in a liposome model system, as measured by the thiobarbituric acid method. Lipid peroxidation was induced by $FeCl_3$ and ascorbic acid, and compared with 2.0 μg Toc. TBARS, thiobarbituric acid-reactive substances; MDA, malondialdehyde; Ext, extracts. □, WEB; ■, HWEB. For other abbreviations see Figure 1.

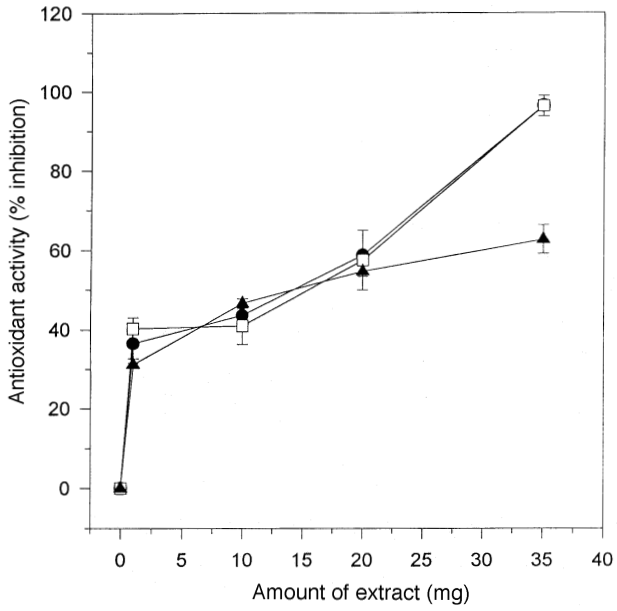

FIG. 3. Inhibitory effects of different amounts of WEB and HWEB on MDA production in rat liver homogenate, induced by $FeCl_2$-ascorbic acid *in vitro,* and compared with Toc. The percentage inhibition of liver tissue peroxidation, 100 − [(absorbance of sample/absorbance of control)] × 100], was calculated to express antioxidant activity. ●, WEB; □, HWEB; ▲, Toc. For abbreviations see Figures 1 and 2.

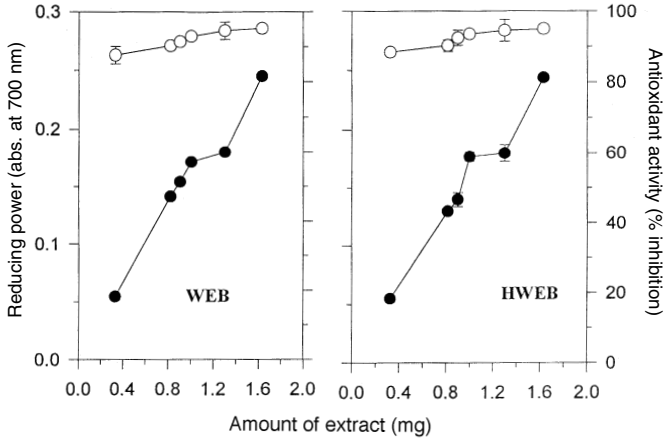

FIG. 4. Reducing power and antioxidant activity of different amounts of WEB and HWEB. The antioxidant activity was determined by the thiocyanate method, and calculation of the percentage inhibition of linoleic acid peroxidation is the same as in Figure 1. ●, Reducing power; ○, antioxidant activity. For abbreviations see Figure 1.

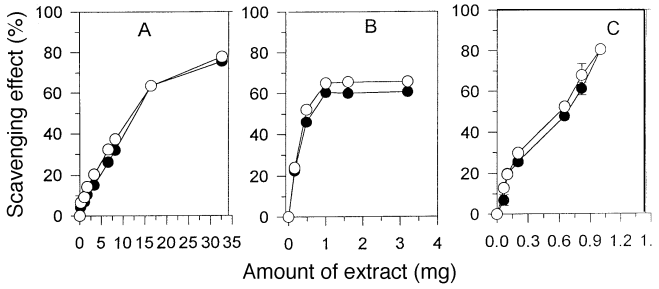

FIG. 5. Scavenging effect of different amounts of WEB and HWEB on the α,α-diphenyl-β-pierylhydrazyl radical (A), superoxide (B), and hydrogen peroxide (C). ●, WEB; ○, HWEB. For abbreviations see Figure 1.

(data not shown), which is a reducing agent and potent reductone (23). Gordon (6) reported that the antioxidant action of reductones is based on breaking the radical chain by donation of a hydrogen atom. Lingnert and Eriksson (24) noted that antioxidative properties are considered to be associated with the presence of reductones. Okuda *et al*. (25) reported that the reducing power of tannins prevents liver injury by inhibiting the formation of lipid peroxides. Reductones are believed not only to react directly with peroxides but also to prevent peroxide formation by reacting with certain precursors. Therefore, extracts of burdock are suggested to act as electron donors, reacting with free radicals to convert them to more stable products, which can terminate radical chain reaction. Our data indicate that the marked antioxidant action of WEB and HWEB may be a result of their reducing power.

Scavenging effects of different amounts of WEB and HWEB on the free radical DPPH and active oxygen, including superoxide and hydrogen peroxide, are shown in Figure 5. The scavenging effect of WEB and HWEB on the DPPH radical increased with increasing amounts of extracts (Fig. 5A). WEB and HWEB (32 mg) both exhibited 80% scavenging activity. The activity of the extracts is therefore attributed to their hydrogen-donating ability (23). It is well known that free radicals cause autoxidation of unsaturated lipids in food (26). On the other hand, antioxidants are believed to intercept the free-radical chain of oxidation and to donate hydrogen from the phenolic hydroxyl groups, thereby forming stable free radicals, which do not initiate or propagate further oxidation of lipids (27). The data obtained reveal that the extracts are free-radical inhibitors and primary antioxidants that react with free radicals. This action may be the main factor to cause inhibition of peroxidation both in linoleic acid and liposome model systems.

The scavenging effect of WEB or HWEB increased with increasing amounts of extract; 60.5% superoxide was inhibited by 1.0 mg of WEB, and 65.0% superoxide was inhibited by 1.0 mg of HWEB (Fig. 5B). Superoxide is a relatively weak oxidant, but it acts on biologically important substances (18). Dahl and Richardson (28) reported that superoxide decomposes to form stronger oxidative species, such as singlet oxygen and hydroxyl radicals, which initiate peroxidation of lipids. Meyer and Isaksen (29) reported that superoxide indirectly initiates lipid peroxidation as a result of superoxide and hydrogen peroxide, which serve as precursors of highly reactive hydroxyl radicals. Robak and Gryglewski (30) reported that antioxidative properties of several flavonoids, such as quercetin, myricetin, and rutin, were a result of scavenging of superoxide. Apparently, the marked antioxidant activity of WEB and HWEB may be related to their high scavenging of superoxide.

WEB and HWEB also were capable of scavenging hydrogen peroxide in a concentration-dependent manner (Fig. 5C). WEB and HWEB at 1.0 mg diminished hydrogen peroxide concentration from 2.0 to 0.39 mM, exhibiting a scavenging effect of 80.5% on hydrogen peroxide; this represented a reduction of 1.61 μmol H_2O_2/mg extract. Hydrogen peroxide itself is rather unreactive, but it can initiate lipid peroxidation or be toxic to cells because it generates hydroxyl radicals by the Fenton reaction (3). Therefore, the ability of WEB and HWEB to scavenge hydrogen peroxide may contribute to their inhibition of lipid peroxidation.

To test the reaction of hydroxyl radical (·OH) with extracts of burdock, the Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \cdot OH$) was used as a source of hydroxyl radical. As shown in Figure 6, the reaction of Fe^{2+} with H_2O_2 in the presence of spin-trapping agent DMPO generated a 1:2:2:1 quartet with hyperfine coupling parameters (a^n and a^h = 14.9 G). Figure 6 shows EPR spectra of DMPO-OH spin adduct in the presence and absence of burdock extracts. WEB and HWEB markedly scavenged the DMPO-OH spin adduct. The EPR signals of DMPO spin adducts formed in the Fenton reaction are presented in Table 2. The addition of WEB and HWEB to the reaction system resulted in dose-dependent inhibition of the EPR signal intensity of DMPO-OH adducts. The hydroxyl radical is an extremely reactive free radical formed in biological systems (18). It can act on and damage almost every molecule found in living cells, such as sugars, amino acids, phos-

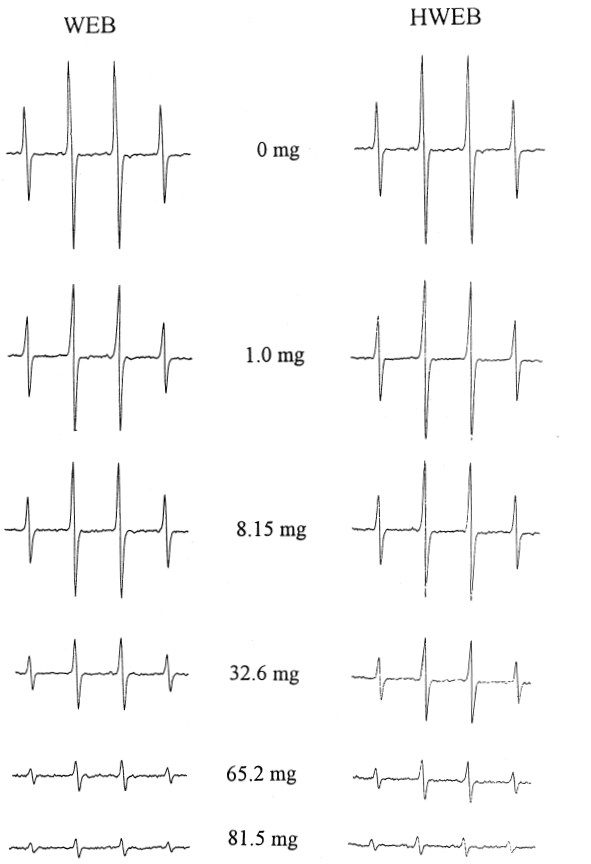

FIG. 6. Electron spin resonance spectrum recorded 2.5 min after mixing in a phosphate buffer solution (pH 7.2) of 55 mM 5,5-dimethyl-pyrroline *N*-oxide with 1.82 mM Fe^{2+}, 1.82 mM H_2O_2, and burdock extracts (WEB or HWEB). Spectrometer settings: receiver gain, 8×10^5; modulation amplitude, 1.0 G; scan time, 200 s; field, 3478.9 ± 50 G; time constant, 0.5 s. For abbreviations see Figure 1.

pholipids, DNA bases, and organic acids (3). Lipid peroxidation is rapidly stimulated by hydroxyl radicals that are sufficiently reactive to abstract hydrogen atoms from unsaturated fatty acids (31).

In the present investigation, WEB and HWEB exhibited marked antioxidant activity. The ability of WEB and HWEB to quench hydroxyl radicals seems to relate directly to the prevention of propagation of the process of lipid peroxidation. WEB and HWEB are both good scavengers of active oxygen species and secondary antioxidants that reduce the rate of chain initiation (6). This observation suggests that burdock drinks (HWEB) prepared from burdock may be healthful. More experiments concerning the nutritional or physiological effects of burdock extracts *in vitro* or *in vivo* are required.

These results clearly indicate that WEB and HWEB show significant antioxidant activity, marked reducing power, and a scavenging effect on free-radical and active oxygen. These properties are chiefly responsible for the antioxidant effect of WEB and HWEB and may in part explain the mechanism of antioxidant activity of burdock extracts.

TABLE 2
Effect of Water Extracts of Burdock (WEB) and Hot Water Extracts of Burdock (HWEB) on EPR Signal Intensity of DMPO-OH Spin Adduct[a]

Extract (mg)	Relative EPR signal intensity (%)	
	WEB	HWEB
0.0	100.0	100.0
1.0	77.7	84.6
8.15	64.4	62.7
32.6	37.8	40.6
65.2	18.7	21.0
81.5	10.4	10.0

[a]EPR spectrum recorded 2.5 min after mixing in a phosphate buffer solution (pH 7.2) of 55 mM DMPO with 1.82 mM Fe^{2+}, 1.82 mM H_2O_2, and burdock extracts (WEB or HWEB). Spectrometer settings: receiver gain, 8×10^5; modulation amplitude, 1.0 G; scan time, 200 s; field, 3478.9 ± 50 G; time constant, 0.5 s. Abbreviations: WEB, water extracts of burdock; HWEB, hot water extracts of burdock; EPR, electron paramagnetic resonance; DMPO, 5,5-dimethyl-pyrroline *N*-oxide.

ACKNOWLEDGMENT

This research work was supported by the National Science Council, Republic of China, under Grant NSC 85-2321-B-041-003.

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[Received March 10, 1997; accepted October 31, 1997]