Anti-allergic Effects of Extracts from Commercial Products of Cooked Burdock

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The anti-allergic effects of commercial products from cooked burdock (burdock salad, fried up burdock and burdock boiled with vinegar) were investigated by analyzing their inhibitory effects on the enzymatic activity (cyclooxygenase-2, lipoxygenase, phospholipase A2 and hyaluronidase) and the suppression of degranulation from rat basophilic leukemia (RBL)-2H3 cells. The methanol extract of burdock boiled with vinegar did not inhibit the cyclooxygenase-2 activity in the experiments. Those of the burdock salad and fried up burdock exhibited inhibitory activities in all the experiments. The burdock salad extract strongly inhibited lipoxygenase, phospholipase A2 and hyaluronidase. Thus, commercial cooked burdock, in addition to raw burdock, has bioactivities corresponding to an anti-allergic effect.

Keywords: cooked burdock, anti-allergic effect, cyclooxygenase, lipoxygenase, phospholipase A2, hyaluronidase, RBL-2H3

Introduction

The components comprising raw burdock (Arctium lappa L.) have shown an anti-oxidative effect (Duh et al., 1998), antimutagenicity (Shinohara et al., 1988), hepatoprotective effect (Lin et al., 2002) and an inhibitory effect on the α-glucosidase activity (Miyazawa et al., 2005).

During manufacturing of commercial burdock products, the raw material which has been washed its surface, peeled its epidermis and cut up is boiled with water, fried up with salad oil, and combined with various ingredients. This process may cause removal or degradation of the active components, such as phenolic compounds, possibly resulting in a commercial burdock product diminishing its bioactivities.

As raw burdock extract possesses an anti-allergic and anti-inflammatory effect (Knipping et al., 2008, Wang et al., 2007), the bioactivities of commercial burdock products (burdock salad, fried up burdock and burdock boiled with vinegar) were investigated for their anti-allergic effects. We evaluated the inhibition of degranulation from rat basophilic leukemia (RBL)-2H3 cells (Bursumian et al., 1981) and the inhibitory effects on the activity of phospholipase A2, cyclooxygenase, lipoxygenase and hyaluronidase, which are involved in iocosanoid (leukotriene and prostaglandin) synthesis and the inflammatory reaction in an allergic response (Funk, 2001; Kakegawa et al., 1985).

Materials and Methods

Materials

Burdock salad was made after the burdock material was boiled for about 5 min, mixed with mayonnaise and other condiments (i.e., sugar, salt, and soy sauce), and then pasteurized at 70°C for 60 min. Fried up burdock (Kinpira in Japanese) was prepared by frying up with salad oil and the condiments for about 5 min. Burdock boiled with vinegar (Tataki) was prepared by boiling with vinegar and the condiments for about 35 min, and then pasteurized at 90°C for 60 min. The commercial products were used as the test samples. Since chlorogenic acid is the main component of burdock polyphenol (Chen et al., 2004) and possesses an anti-allergic effect (Shimoda et al., 2006), its commercial preparation (Sigma-Aldrich, Inc., St. Louis, MO, USA) was used as the reference phenolic compound. Epigallocatechin gallate (EGCg, LKT Laboratories, Inc., St. Paul, MN, USA) was used as a typical natural inhibitor. To compare the three commercial products, raw burdock was also used as the control.

Methanol Extraction and Sample Preparation

Burdock salad, Kinpira and Tataki were washed with tap water to remove the added condiments and then extracted in methanol according to Hikawa et al. (1991) with some modifications. Briefly, 10 g of each minced burdock samples was extracted with 40 mL methanol by agitation using a magnetic hot stir-
rer for 30 min. After filtration through No. 2 filter paper (Toyo Roshi Kaisha, Ltd., Tokyo, Japan), the residues were extracted twice with 40 mL of 80% methanol for 30 min. After filtration through the filter paper, the resulting extracts were concentrated using a rotary evaporator at 40°C to about one-third their original volume. The concentrates were then centrifuged at 17400 × g for 10 min, and the supernatants were applied to a column (2.0 i.d. × 15 cm) of ODS (Chromatorex ODS, DM 1020T, Fuji Silysis Chemical, Ltd., Kasugai, Aichi, Japan). After washing the column with water, the adsorbates were eluted with methanol.

Measurement of Phenolic Compound Content The amount of phenolic compounds in the methanol extracts was measured by the Folin-Denis method (Folin and Denis, 1915). Concentrations of the samples are expressed as the amount of their phenolic compounds.

Assay of Degranulation from RBL-2H3 Cells

i) Cell culture RBL-2H3 cells provided by the Health Science Research Resources Bank (JCRB0023, Sennan, Osaka, Japan) were cultured in Eagle’s Modified Essential Medium (EMEM, Sigma) supplemented with fetal bovine serum (final, 10%; Lot, 915427; Biological Industry, Ltd., Osaka, Japan), penicillin (final, 100000 U/L; Sigma-Aldrich) and streptomycin (final, 100 mg/L; Sigma). The cells were precultured and sensitized with anti-DNP IgE (0.2 μg/well; Sigma) overnight. After removal of anti-DNP IgE, each test sample was added, and the cells were incubated for 10 min. Subsequently, the cells were stimulated with DNP-BSA (4 μg/well; LSL Co., Ltd., Tokyo, Japan) for 30 min. Degranulation from the RBL cells was determined by measuring the activity of released β-hexosaminidase, a marker of degranulation (Schwartz et al., 1981), as described previously (Sugiura et al., 2007). The β-hexosaminidase activity was not inhibited by any of the samples. The inhibition ratio was calculated and compensated for by subtracting the colorization of the sample solution.

Inhibitory Effects on Enzymatic Activities Involved in Allergic Reaction

i) Phospholipase A2 The inhibitory effects of the methanol extracts and typical active compounds on the phospholipase A2 (PLA2) activity was analyzed using the methods of Shibata et al. (2003) with some modifications. Briefly, diheptanoyl thio-PC (Cayman Chemical Co., Ann Arbor, MI, USA) dissolved in DMSO was diluted with 25 mM Tris-HCl buffer (pH 7.5) containing 10 mM CaCl2, 100 mM KCl, 0.3 mM Triton-X 100 and 1 mg/mL of BSA. The final concentration of the diluted solution was 1.66 mM. Dihexanoyl thio-PC solution (200 μL) was added to 5 μL of each sample or DMSO (control), followed by the addition of 10 μL of 5,5’-dithio-bis-(2-nitrobenzoic acid) (50 mM; DTNB; Sigma-Aldrich) diluted with the Tris-HCl buffer. After storage of the reaction mixture at room temperature for 5 min, the background absorbance at the optical density of 405 nm (OD405) was measured using a spectrophotometer (MPR-A4i II, Tosoh Corp., Tokyo, Japan). Porcine pancreatic PLA2 (10 μL; 10 units; Sigma-Aldrich) was then immediately added to start the enzymatic reaction. After 1.5 min from the addition of the PLA2, the change in the OD405 value was measured. The inhibition ratio of the PLA2 activity was calculated using the following formula:

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\text{Inhibition ratio (\%)} = \left[1 - \left(\frac{C_{1.5} - T_0}{C_0 - T_0}\right)\right] \times 100
\]

where \(C_0\) is the OD405 without the sample before PLA2 addition; \(C_{1.5}\) is the OD405 without the sample after 1.5 min; \(T_0\) is the OD405 with the sample before PLA2 addition, and \(T_{1.5}\) is the OD405 with the sample after 1.5 min.

ii) Cyclooxygenase-2 The inhibitory effects of the methanol extracts and typical active compounds on allergy-induced cyclooxygenase-2 (COX-2) (Funk, 2001) activity were investigated using a commercial ELISA kit for cyclooxygenase inhibitor screening (Cayman) according to the manufacturers’ protocols.

iii) Soybean lipoxigenase Soybean lipoxigenase (SBL, Type I-B, Sigma-Aldrich) was used as an alternative for the human 5-lipoxygenase (Komoda et al., 1995). The inhibitory effects of the methanol extracts and typical active compounds on the SBL activity were evaluated according to Komoda et al. (1995).

iv) Hyaluronidase The inhibitory effect on the hyaluronidase (HA) activity was measured using the method of Kakegawa et al. (1985).

Results and Discussion

As shown in Table 1, the tested samples suppressed the degranulation from the RBL cells. EGCg exhibited the strongest inhibition. Chlorogenic acid exhibited the second strongest inhibition. In the methanol extracts from the commercial burdock products, the inhibition of the Kinpira extract was the strongest, while that of burdock salad was the weakest. The raw burdock and tataki extracts exhibited moderate inhibition.

The inhibitory effects of each sample on the enzymatic activities (PLA2, COX-2, SBL and HA) are shown in Table...
The burdock salad, Kinpira, and raw burdock extracts, as well as EGCg, inhibited the four enzymatic activities; the inhibition by the burdock salad extract was significant. The inhibition ratios for the PLA2 activity of the raw burdock extract and chlorogenic acid (2 mg/mL) were 38.1% and 21.0%, respectively, while those for the HA activity of Tataki extract and chlorogenic acid were 18.6% and 10.6%, respectively. The Tataki extract and chlorogenic acid showed no inhibition on the COX-2 activity at all tested concentrations.

The methanol extracts of the tested commercial products showed varying levels of degranulation suppression in the RBL cells (Table 1). Among the extracts, differences in the inhibitory effects on the four enzymatic activities occurred (Table 2). Thus, it is found that the difference in the inhibitory effect of the extract was due to characteristic of the manufacturing process. The difference in the inhibitory effect may be attributed to the interactions of various components, altered through a complex manufacturing process and interaction with the condiments. For example, the methanol extract of Tataki did not inhibit the COX-2 activity in present study (Table 2). Since the heating characteristics of the Tataki manufacturing process are different from the burdock salad and Kinpira, the active compound in the Tataki extract may become unable to inhibit the COX-2 activity by heating. From the present results that the extract of Tataki and chlorogenic acid weakly inhibited the HA activity (Table 2), the active compound responsible for HA inhibition may not be resistant to a long heating as in the Tataki manufacturing process, and the HA inhibition activity may be attributed to not only one compound such as chlorogenic acid, but a synergy between various active compounds. The inhibitory effect of the burdock salad extract on the four enzymatic activities tended to be stronger compared to that of the Kinpira, Tataki and raw burdock extract, also suggesting a synergic effect among various components altered by the burdock salad manufacturing process. Further studies on the alternation of the inhibitory effect by a synergic effect and interaction among components are required.

Edible burdock includes chlorogenic acid, caffeic acid and caffeic acid derivatives as the phenolic compounds (Chen et al., 2004; Maruta et al., 1995). As phenolic compounds express anti-allergic and anti-inflammatory effects (Kimura and Okuda, 1987; Shimoda et al., 2006; Song et al., 2008), they may be the active compounds in the burdock methanol extracts.

In this study, the methanol extracts from the commercial products of cooked burdock exhibited anti-allergic effects in vitro. To demonstrate the availability of the commercial products in humans, further studies involving these active compounds and in vivo experiments are required.

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References


