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Various Biological Activities of Ramie (*Boehmeria nivea*)

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Abstract The purpose of this study was to evaluate the biological activities of extracts of ramie (*Boehmeria nivea* (L.) Gaud.), hereafter referred to as Bn. Bn extracts from various collecting area were extracted with methanol. Two extracts from our study, Bn-13 and -82, showed significant antioxidant properties, likely due to their ability to scavenge free radicals. In addition, Bn extracts showed stronger anti-bacterial activity against *Escherichia coli* (Bn-40), *Stapylococcus aureus* (Bn-33), and *Helicobacter pylori* (Bn-05). In addition, this study was conducted to evaluate the anti-inflammatory effects of Bn extracts in lipopolyssacharide (LPS)- and interferon-γ (IFN-γ)-stimulated RAW 264.7 macrophages cells. Bn-37 significantly inhibited the production LPS/IFN-γ-induced nitric oxide. The most noteworthy anti-cancer effect was found in Bn-23. Bn-08 showed inhibition of aldose reductase. This study provides basic information for the development of functional foods.

Keywords biological activity · *Boehmeria nivea* · functional food · ramie

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Introduction

Ramie (*Boehmeria nivea* (L.) Gaud.) is a perennial herbaceous plant of the Urticaceae family and it is mainly planted in Asian countries including China, Korea, the Philippines, and India (Liu et al., 2001). Ramie is commonly referred to as China grass, white ramie, green ramie, or rhea, and was used in mummy cloths in Egypt during the period 5,000–3,300 BC. Furthermore, it is known have to the strongest natural vegetative fiber and is an important textile material, because it is extremely absorbent, dries quickly, dyes fairly easily, and is unusually tolerant of bacteria, mildew, and insect attacks (Wang et al., 2007; 2008). Because it is known that the green leaves of ramie are rich in nutrients such as vitamins, minerals, and various bioactive materials, there have been many studies examining its use in teas and health foods (Gupta and Wagle, 1988). As a natural herbal resource, ramie has various functions. It has been used in traditional medicine for diuretic and antipyretic purposes (Lin et al., 1997). In Korea, there have been some studies on the use of its smooth leaves in foods such as tteok (traditional Korean rice cakes) (Kim et al., 1993). Recently, ramie was shown to protect against hepatotoxicity, eliminate inflammation, neutralize poison, and dissipate heat (Lin et al., 1998). However, the biological activities of ramie are not well known.

Free radical damage is one of the major processes that contribute to degenerative diseases associated with aging including cancer, cardiovascular disease, immune-system decline, brain dysfunction, and cataracts (Ames et al., 1993). Therefore, we evaluated anti-oxidant activity of ramie using different treatments such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl (·OH) radical.

Furthermore, we examined the anti-bacterial activity of ramie against *E. coli*, *S. aureus*, and *H. pylori.* The effects of ramie with respect to inflammation and cancer were evaluated using RAW 264.7 macrophage cells and adenocarcinoma gastric stomach (AGS) cells, respectively. In addition, inhibitory activities of ramie on aldose reductase (AR) were investigated. The objective of the current study is focus on the investigation of biological activity of Bn extracts from various collecting area.

Materials and Methods

Plant materials. Ramie (*Boehmeria nivea* (L.) Gaud.) was collected by the staff of Yeong-Gwang Agricultural Technology Center, Korea. The collection areas of Bn extracts are shown in Table 1.

Instruments and reagents. An evaporator was obtained from EYELA (Japan) and methanol was purchased from Sam Chun Pure Chemical Co. (Pyeongtaek, Korea). DPPH and 2-deoxyribose used to investigate radical-scavenging activity were obtained from Sigma Chemical Co. (USA), and hydrogen peroxide (H_2O_2) was purchased from Junsei Chemical Co. (Japan). AGS and RAW 264.7 cells were obtained from the Korea Cell Line Bank (KCLB, Korea). Dulbecco's Modified Eagle Medium (DMEM), Roswell Park Memorial Institute 1640 (RPMI-1640), fetal bovine serum (FBS), and penicillin/streptomycin were obtained from Welgene (Korea). The lipopolysaccharide (LPS) used in this study was from Sigma Chemical Co. and interferon-gamma (IFN-γ) was from Pepro Tech (NJ, USA). The Griess reagent, 3-(4,5-dimethylthiazol-2-yl)-2,3-diphenyl tetrazolium bromide (MTT), 3,3-tetramethylene glutaric acid (TMG), and dimethyl sulfoxide (DMSO) were obtained from Sigma Chemical Co.

Preparation of Bn methanol (MeOH) extracts. Ten grams of dried Bn extracts were extracted with MeOH (200 mL ×3) under reflux conditions and the solvent was evaporated *in vacuo*. Each individual MeOH extract (1.0 mg) was dissolved in DMSO (5 μL).

DPPH and ·OH radical-scavenging activity. In a 96 micro-well plate, 100 μL of each sample was added to an ethanol solution of DPPH (100 μL) according to the method detailed by Hatano et al. (1989). After vortexing, the mixture was incubated for 30 min at room temperature and absorbance was measured at 540 nm. The DPPH radical-scavenging activity was recorded as a percentage (%) compared to the control. Scavenging of ·OH radicals was measured according to the method given by Chung et al. (1997). The reaction mixture contained 10 mM $FeSO_4 \cdot 7H_2O_2$-EDTA, 10 mM 2-deoxyribose, and the sample solutions. After incubation at 37°C for 4 h, the reaction was stopped by adding a 2.8% trichloroacetic acid and 1.0% thiobarbituric acid solution. The solution was boiled for 20 min and then cooled in a water bath. ·OH scavenging activity was measured at 490 nm.

Anti-bacterial activity. *E. coli* and *S. aureus* were provided by Korean Culture Center of Microorganisms (KCCM, Korea). Trypticase soy agar (TSA) was purchased from BD Difco (USA), and disc paper was obtained from Advantec (Japan). The TSA culture medium contained pancreatic casein digest (15 g), papaic soybean digest (5 g), NaCl (5 g), sodium chloride (15 g), and agar (15 g) in distilled water (1 L). Microaerophilic conditions were maintained at 37°C. *H. pylori*, provided by Korean Type Culture Collection (KTCC, Daejeon, Korea), were cultured in Brucella broth (Difco, NJ, USA) containing 10% horse serum (Welgene) and, for testing, were grown on a medium prepared with (per liter) BD Bacto dextrose (1 g), BD Bacto yeast extract (2 g) (Becton, Dickinson and Company [BD], Franklin Lakes, USA), sodium chloride (5 g), and sodium bisulfate (0.1 g). Anti-bacterial activity against *S. aureus*, *E. coli*, and *H. pylori* was measured by the disc agar method (Davidson and Parish, 1989). Plates of medium were spread with 0.1 mL of culture broth, and 15 μg/30 μL of the fractions and compounds were pipetted onto sterile filter paper discs (8 mm). Inhibition zones were determined after 24 h at 37°C.

Cell culture. AGS cells were maintained in RPMI-1640 medium and RAW 264.7 cells were cultured in DMEM containing 100 U/mL of penicillin/streptomycin and 10% FBS at 37°C in a 5% CO_2 incubator. Cells were sub-cultured weekly with 0.05% trypsin-EDTA in phosphate buffered saline.

Cell viability assay. After confluence had been reached, the cells were plated at a density of 5×10^4 cells/well into 24 well plates, incubated for 2 h, and then treated with LPS (1 μg/mL) and IFN-γ (10 ng/mL). Samples were treated for 24 h. After incubation, cell viability was determined using the MTT assay. MTT solution was added to each 24-well plate, the plates were incubated for 4 h at 37°C, and the medium containing MTT was removed. The incorporated formazan crystals in the viable cells were solubilized with 1 mL of DMSO and the absorbance of each well was read at 540 nm (Mosmann, 1983).

Measurement of nitrite. Nitric oxide (NO) production was assayed by measuring the accumulation of nitrite using a microplate assay method based on the Griess reaction (Sreejayan and Rao, 1997). RAW 264.7 cells were seeded in 24-well plates (5×10^4 cells/well) to which LPS (1 μg/mL) and IFN-γ (10 ng/mL) were added. After incubating the samples for 24 h, 100 μL of culture supernatant was allowed to react with 100 μL of Griess reagent in 96-well plates and the mixture was incubated at room temperature for 15 min. The optical density of the samples was measured at 540 nm using a microplate reader (Chiou et al., 1997).

Inhibition of AR. Rat lenses (one lens per 0.5 mL of sodium buffer) were removed from Sprague-Dawley rats (weighing 250–280 g) and preserved until use by freezing. The rat lenses were homogenized and centrifuged at 10,000 rpm (4°C, 20 min) and the supernatant was used as an enzyme source. AR activity was spectrophotometrically determined by measuring the decrease in β-NADPH absorption at 340 nm. Absorbance measurements were obtained for a 4 min period at room temperature in a quartz cell with DL-glyceraldehyde as the substrate (Sato and Kador, 1990; Mok et al., 2012). The assay mixture contained 0.1 M potassium phosphate buffer (pH 7.0), 0.1 M sodium phosphate buffer (pH 6.2), 1.6 mM β-NADPH, and the test samples (in DMSO), with 0.025 M DL-glyceraldehyde as the substrate.

Statistical analysis. Results are expressed as means±SD.

Table 1 The collection areas of Bn

Sample	Collection area	Sample	Collection area	Sample	Collection area	Sample	Collection area	Sample	Collection area
Bn-01	Improved Variety in Duam, Gwangju	Bn-19	Local Variety in Hyeonsan, Haenam	Bn-37	Local Variety in Seocheon-3	Bn-55	Local Variety in North Jeju	Bn-73	Natural cross of White Peel Variety
Bn-02	Seobang Variety in Hansan, Seocheon	Bn-20	Local Variety inside Wando-gun	Bn-38	Local Variety in Seocheon-4	Bn-56	Ramie in Gwangyang	Bn-74	Natural cross of White Peel Variety
Bn-03	Local Variety in Duwon, Goheung	Bn-21	Local Variety outside Wando-gun	Bn-39	Local Variety in Seocheon-5	Bn-57	Ramie in Hoecheon, Boseong	Bn-75	Natural cross of White Peel Variety
Bn-04	White Peel Variety in Gwayeok, Gwangju	Bn-22	Local Variety in Daeya, Wando	Bn-40	White Peel Variety in Biin, Seocheon-1	Bn-58	White Peel Variety in Hansan, Seocheon	Bn-76	Natural cross of White Peel Variety
Bn-05	Seobang Variety in Hansan, Seocheon	Bn-23	Local Variety in Hakgyo, Hampyeong	Bn-41	White Peel Variety in Biin, Seocheon-2	Bn-59	Biin, Seocheon , Duam, Gwangju	Bn-77	Natural cross of Taiwan Variety
Bn-06	Seobang Variety in Seocheon Agricultural Technology center	Bn-24	Local Variety in Gwansan, Jangheung	Bn-42	Local Variety in Sagok, Gongju	Bn-60	Natural cross of Improved Variety in Duam	Bn-78	Local Variety in Worya, Hampyeong
Bn-07	Taiwan Variety in Duwon, Goheung	Bn-25	Local Variety in Mokcheon, Gangjin	Bn-43	Local Variety in Yugu, Gongju	Bn-61	Natural cross of Improved Variety in Duam	Bn-79	Local Variety in Yucheon, Buan
Bn-08	Local Variety in Goheung-eup	Bn-26	Local Variety in Hoecheon, Boseong	Bn-44	Local Variety in Gyosa-ri, Goseong-eup	Bn-62	Natural cross of Improved Variety in Duam	Bn-80	Local Variety in Hansan, Seocheon-3
Bn-09	Taiwan Variety in Geumsan, Goheung	Bn-27	Local Variety in Beolgyo, Boseong	Bn-45	Improved Variety in Bongsu, Uiryeong	Bn-63	White Peel Variety in Taiwan, Local Variety in Jeomam	Bn-81	Local Variety in Hansan, Seocheon-4
Bn-10	Ramie in Mangun, Muan	Bn-28	Local Variety in Deungnyang, Boseong	Bn-46	Local Variety in Yangwon, Cheongdo	Bn-64	White Peel Variety in Taiwan, Local Variety in Jeomam	Bn-82	Local Variety in Hansan, Seocheon-5
Bn-11	Local Variety in Bugil, Jangseong	Bn-29	Local Variety in Deokheung, Goheung	Bn-47	Local Variety in Iseo, Cheongdo	Bn-65	White Peel Variety in Taiwan, Local Variety in Jeomam	Bn-83	Local Variety in Gwanchon, Imsil
Bn-12	Local Variety in Dasi, Naju	Bn-30	Local Variety in Gochang-eup-1	Bn-48	Local Variety in Gwangdo, Tongyeong-1	Bn-66	White Peel Variety in Taiwan, Local Variety in Jeomam	Bn-84	Local Variety in Namwon
Bn-13	Local Variety in Unam, Gwangju	Bn-31	Local Variety in Gochang-eup-2	Bn-49	Local Variety in Gwangdo, Tongyeong-2	Bn-67	Improved Variety in Gwangju , Taiwan Variety in Goheung	Bn-85	Local Variety in Yaro, Hapcheon
Bn-14	Local Variety in Jeomam, Goheung	Bn-32	Local Variety in Sangseo, Buan-1	Bn-50	Local Variety in Namhae-eup	Bn-68	Improved Variety in Gwangju, White Peel Variety in Gwayeok	Bn-86	Local Variety in Myosan, Hapcheon
Bn-15	White Peel Variety in Nokdong-gil, Gwangju	Bn-33	Local Variety in Sangseo, Buan-2	Bn-51	Local Variety in Jeongnyang, Hadong	Bn-69	Improved Variety in Gwangju, White Peel Variety in Gwayeok	Bn-87	Local Variety in Bongcheon, Hadong
Bn-16	Local Variety in Bongsan	Bn-34	Local Variety in Byeoksan, Gimje	Bn-52	Local Variety in Jangseungpo	Bn-70	Improved Variety in Gwangju, White Peel Variety in Gwayeok	Bn-88	Local Variety in Jukgok, Gokseong
Bn-17	Local Variety in Dasi, Naju	Bn-35	Local Variety in Jugok, Jinan	Bn-53	Local Variety in Chungmusi	Bn-71	Natural cross of Seobang Variety	Bn-89	Local Variety in Seokgok, Gokseong
Bn-18	Local Variety in Baegya-gil, Haenam	Bn-36	Local Variety in Hansan-1	Bn-54	Local Variety in Geoje, Gyeongsangnam-do	Bn-72	Natural cross of White Peel Variety	Bn-90	Local Variety in Baeksu, Yeonggwang

Results and Discussion

Anti-oxidant activities. Oxidative stress, induced by free radicals, is a primer factor in various degenerative diseases. Reactive oxygen species (ROS) in the form of superoxide anion, ·OH, and H_2O_2 are generated by normal metabolic processes. These ROS are capable of damaging a wide range of essential biomolecules (Halliwell et al., 1992). DPPH is usually used to evaluate free radical scavenging

Table 2 Anti-oxidative activities of Bn extracts

Sample	Radical scavenging activity (%)		Sample	Radical scavenging activity (%)		Sample	Radical scavenging activity (%)	
	DPPH	·OH		DPPH	·OH		DPPH	·OH
Bn-01	84.91±0.06	97.87±0.07	Bn-31	78.21±0.50	90.22±0.13	Bn-61	79.66±0.36	98.78±0.12
Bn-02	86.36±0.11	93.71±0.12	Bn-32	82.76±0.20	93.62±0.01	Bn-62	53.74±0.16	94.34±0.08
Bn-03	59.71±1.81	75.69±0.04	Bn-33	68.55±0.04	93.08±0.06	Bn-63	80.21±0.18	91.91±0.13
Bn-04	84.35±0.49	94.56±0.11	Bn-34	81.23±0.93	91.02±0.03	Bn-64	52.65±0.48	95.80±0.26
Bn-05	81.49±0.36	94.59±0.34	Bn-35	51.90±0.34	91.06±0.06	Bn-65	67.28±0.34	99.54±0.14
Bn-06	76.28±0.35	96.93±0.02	Bn-36	83.02±0.25	91.93±0.08	Bn-66	67.63±0.35	87.83±0.11
Bn-07	80.69±0.16	98.03±0.16	Bn-37	81.29±0.45	99.39±0.47	Bn-67	55.76±0.11	98.09±0.23
Bn-08	78.30±0.42	90.15±0.19	Bn-38	81.69±0.11	96.85±0.40	Bn-68	60.43±0.16	94.58±0.13
Bn-09	75.66±0.08	98.32±0.49	Bn-39	85.33±0.68	92.20±0.13	Bn-69	78.66±0.23	96.46±0.04
Bn-10	81.40±0.10	97.00±0.02	Bn-40	88.25±1.02	94.74±0.08	Bn-70	86.62±0.29	94.54±0.70
Bn-11	76.45±0.25	98.81±0.37	Bn-41	73.89±0.72	98.64±0.85	Bn-71	75.06±0.02	99.20±0.09
Bn-12	82.68±0.18	97.53±0.16	Bn-42	50.67±0.54	99.04±0.07	Bn-72	81.43±0.28	93.35±0.42
Bn-13	95.17±0.04	91.32±0.28	Bn-43	54.25±0.12	93.40±0.04	Bn-73	77.51±0.32	99.08±0.28
Bn-14	83.57±0.11	93.87±0.15	Bn-44	45.53±1.13	93.37±0.01	Bn-74	81.22±0.01	95.92±0.28
Bn-15	71.64±1.08	93.94±0.02	Bn-45	76.99±0.13	98.87±0.01	Bn-75	64.76±0.26	92.30±0.07
Bn-16	74.07±0.06	93.49±0.08	Bn-46	43.86±0.33	98.09±0.60	Bn-76	88.30±0.28	95.10±0.01
Bn-17	80.13±0.40	89.77±0.20	Bn-47	37.65±0.95	89.65±0.16	Bn-77	79.33±0.35	92.57±0.14
Bn-18	84.10±0.09	93.37±0.20	Bn-48	77.30±0.74	89.87±0.16	Bn-78	77.37±0.14	99.08±0.21
Bn-19	79.19±0.04	91.69±0.46	Bn-49	82.30±0.40	91.03±0.52	Bn-79	76.08±0.10	97.83±1.46
Bn-20	86.52±0.27	94.58±0.97	Bn-50	86.50±0.04	97.56±0.40	Bn-80	82.63±0.28	99.08±1.24
Bn-21	87.40±0.82	92.98±0.21	Bn-51	64.47±0.61	91.54±0.21	Bn-81	78.94±0.01	98.77±0.44
Bn-22	85.45±0.03	93.73±0.03	Bn-52	55.78±0.18	91.04±0.66	Bn-82	90.71±0.07	96.57±1.10
Bn-23	89.24±0.18	94.36±0.01	Bn-53	21.01±0.31	91.11±0.42	Bn-83	78.50±0.16	94.22±1.33
Bn-24	65.98±0.10	95.05±0.55	Bn-54	78.43±0.48	92.31±0.65	Bn-84	44.36±0.24	97.84±0.13
Bn-25	73.67±0.03	93.87±0.21	Bn-55	77.72±0.27	96.01±0.57	Bn-85	62.36±0.42	98.71±1.44
Bn-26	83.37±0.12	89.20±0.02	Bn-56	66.53±0.52	97.18±1.03	Bn-86	33.90±0.04	90.76±0.28
Bn-27	88.78±0.34	98.17±0.06	Bn-57	56.63±0.24	88.61±0.24	Bn-87	37.50±0.62	90.89±1.22
Bn-28	70.98±0.00	98.96±0.45	Bn-58	36.33±0.10	89.74±0.66	Bn-88	55.49±0.25	94.40±0.13
Bn-29	84.31±0.03	95.31±0.06	Bn-59	71.38±0.32	92.47±0.13	Bn-89	53.66±0.17	90.67±0.28
Bn-30	54.68±1.26	96.30±0.31	Bn-60	76.09±1.00	90.59±0.01	Bn-90	65.43±0.23	85.82±0.87

activity of antioxidants (Oyaizu, 1986). DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares et al., 1997). In a previous study, ramie leaf powder was shown to have high free radical scavenging ability (Park et al., 2011). The scavenging effect of Bn extracts with DPPH radical is shown in Table 2. Among Bn extracts, 34 kinds of Bn extracts are responsible for more than 80% of the observed radical scavenging activity at a concentration of 100 μg/mL. In particular, Bn-13 and -82 showed a higher capacity than the other extracts, showing 95.17 and 90.71%, respectively. Also, the result of ·OH radical scavenging activity demonstrated that all Bn extracts showed over 80% scavenging activity. The results from radical scavenging systems reveal that Bn extracts have a significant anti-oxidant effect.

Anti-bacterial activities. *S. aureus* and *E. coli* are considered important causes of disease in the world according to sources that report on food-poisoning issues (Zhang et al., 1998; Rauha et al., 2000). Controlling the numbers and growth of *S. aureus* and *E. coli* is important in the food industry. *H. pylori*, a Gram-negative bacterium, plays a role in a variety of gastric diseases including chronic gastritis, peptic ulcer, and gastric cancer (Forman et al., 1991). The anti-bacterial activities of Bn extracts against *E. coli, S. aureus*, and *H. pylori* are shown in Table 3. Our results represent the anti-bacterial effects of Bn extracts at a concentration of 15 μg/30 μL. The result of anti-bacteria activity against *E. coli*, a Gram negative bacteria, demonstrated that the following-11 Bn extracts (Bn-06, -10, -15, -17, -25, -29, -39, -40, -41, -43, and -60) showed growth inhibition zones greater than 15 mm. Meanwhile, seven kinds of Bn extracts (Bn-03, -16, -25, -33, -35, -38, and -41) produced inhibition zones of *S. aureus* greater than 15 mm. Specifically, Bn-40 produced the largest growth inhibition zone against *E. coli* and Bn-33 produced the largest growth inhibition zone against *S. aureus*, showing 18 and 19 mm, respectively. The result for *H. pylori*, which causes gastritis and gastric cancer, includes seven Bn extracts (Bn-03, -05, -21, -37, -38, -45, and -61) that produced zones of *H. pylori* inhibition greater than 12-14 mm. Particularly, Bn-05 significantly inhibited growth of *H. pylori* (14 mm).

Anti-inflammatory activity. LPS is a cell wall component of all Gram-negative bacteria. LPS-induced activation of macrophages

Table 3 Anti-bacterial activities of Bn extracts

Sample (15 μg/30 μL)	Clear zone (mm)			Sample (15 μg/30 μL)	Clear zone (mm)		
	E. coli	*S. aureus*	*H. pylori*		*E. coli*	*S. aureus*	*H. pylori*
Bn-01	14.0±0.7	14.0±2.1	11.0±0.7	Bn-46	12.0±3.5	11.0±1.4	11.0±0.7
Bn-02	13.0±1.4	14.0±2.8	11.0±2.1	Bn-47	11.0±2.1	12.0±0.0	10.0±0.7
Bn-03	13.0±0.0	15.0±0.7	12.0±0.7	Bn-48	10.0±1.4	12.0±0.0	10.0±0.7
Bn-04	12.0±1.4	12.0±0.0	-	Bn-49	10.0±1.4	-	9.0±0.0
Bn-05	14.0±1.4	14.0±0.7	14.0±3.5	Bn-50	11.0±0.7	13.0±2.8	-
Bn-06	17.0±0.7	11.0±1.4	11.0±0.7	Bn-51	-	-	9.0±0.7
Bn-07	14.0±0.7	11.0±1.4	-	Bn-52	9.0±1.4	11.0±2.1	-
Bn-08	12.0±0.0	11.0±2.1	-	Bn-53	10.0±1.4	12.0±3.5	-
Bn-09	10.0±0.0	14.0±2.8	-	Bn-54	13.0±1.4	14.0±0.0	-
Bn-10	15.0±0.7	11.0±0.7	10.0±0.0	Bn-55	14.0±0.7	13.0±0.7	-
Bn-11	11.0±0.7	10.0±1.4	-	Bn-56	11.0±2.1	10.0±0.0	10.0±0.7
Bn-12	14.0±2.1	11.0±2.1	-	Bn-57	-	9.0±0.0	10.0±0.7
Bn-13	10.0±0.7	9.0±0.0	-	Bn-58	-	12.0±0.0	9.0±0.0
Bn-14	13.0±5.0	13.0±0.7	-	Bn-59	12.0±0.7	14.0±0.7	10.0±1.4
Bn-15	16.0±3.5	13.0±1.4	-	Bn-60	17.0±6.4	12.0±3.5	11.0±0.7
Bn-16	11.0±0.0	16.0±0.7	-	Bn-61	-	13.0±0.7	13.0±3.5
Bn-17	15.0±0.7	13.0±0.7	11.0±0.7	Bn-62	10.0±0.0	12.0±0.0	-
Bn-18	13.0±0.0	11.0±0.0	9.0±0.0	Bn-63	14.0±6.4	11.0±0.0	-
Bn-19	12.0±0.0	10.0±0.0	-	Bn-64	10.0±0.0	11.0±1.4	-
Bn-20	10.0±1.4	10.0±0.7	-	Bn-65	10.0±1.4	14.0±0.0	10.0±0.0
Bn-21	12.0±2.1	12.0±0.7	12.0±0.7	Bn-66	10.0±0.0	12.0±0.0	10.0±0.0
Bn-22	13.0±0.7	10.0±0.0	11.0±0.0	Bn-67	13.0±2.1	13.0±0.7	-
Bn-23	14.0±0.7	12.0±0.7	10.0±1.4	Bn-68	12.0±0.7	11.0±0.0	10.0±0.0
Bn-24	12.0±0.0	13.0±1.4	10.0±0.0	Bn-69	12.0±0.7	13.0±1.4	11.0±1.4
Bn-25	16.0±0.0	15.0±0.0	-	Bn-70	-	13.0±0.7	11.0±0.7
Bn-26	14.0±0.7	14.0±0.7	-	Bn-71	-	12.0±0.0	11.0±0.7
Bn-27	14.0±0.7	11.0±0.0	9.0±0.7	Bn-72	10.0±0.7	-	10.0±0.7
Bn-28	12.0±0.7	11.0±2.1	-	Bn-73	-	-	10.0±0.7
Bn-29	16.0±0.7	13.0±0.0	10.0±1.4	Bn-74	-	-	10.0±0.7
Bn-30	14.0±2.8	12.0±1.4	10.0±0.7	Bn-75	11.0±2.1	12.0±0.7	10.0±1.4
Bn-31	13.0±0.7	11.0±1.4	-	Bn-76	10.0±0.7	11.0±0.7	10.0±1.4
Bn-32	13.0±0.0	12.0±3.5	-	Bn-77	-	12.0±1.4	-
Bn-33	12.0±0.0	19.0±0.0	-	Bn-78	-	10.0±0.7	10.0±0.0
Bn-34	12.0±0.0	12.0±0.0	-	Bn-79	10.0±0.7	14.0±2.1	10.0±0.7
Bn-35	13.0±1.4	17.0±1.4	-	Bn-80	-	13.0±2.1	-
Bn-36	11.0±0.0	13.0±0.0	-	Bn-81	11.0±0.0	12.0±0.0	10.0±0.0
Bn-37	14.0±6.4	14.0±1.4	13.0±2.8	Bn-82	11.0±0.0	11.0±1.4	10.0±0.0
Bn-38	13.0±5.0	17.0±1.4	12.0±0.7	Bn-83	-	13.0±0.0	10.0±0.0
Bn-39	15.0±0.7	13.0±0.0	-	Bn-84	-	12.0±0.0	10.0±0.7
Bn-40	18.0±2.8	12.0±0.0	10.0±0.7	Bn-85	11.0±0.7	12.0±0.0	9.0±0.0
Bn-41	15.0±0.0	15.0±0.0	10.0±0.7	Bn-86	-	13.0±2.1	9.0±0.0
Bn-42	12.0±0.7	13.0±0.7	10.0±0.7	Bn-87	-	-	-
Bn-43	15.0±0.7	12.0±0.7	-	Bn-88	-	11.0±0.0	-
Bn-44	14.0±2.1	14.0±0.7	-	Bn-89	-	-	-
Bn-45	11.0±2.1	13.0±0.7	12.0±1.4	Bn-90	-	-	-

led to production of bioactive lipids, ROS, and in particular, inflammatory cytokines (Beutler and Rietschel, 2003). LPS and IFN-γ can synergistically stimulate cells to produce a large amount of NO (Nathan, 1992). The synthesis of NO, a mediator of inflammatory function, was greatly increased when RAW 264.7 cells were co-stimulated with LPS and IFN-γ. Increased NO production is a common phenomenon that occurs in LPS/IFN-γ-stimulated macrophages and is used as an indicator of inflammatory response. As shown in Table 4, when Bn extracts were added to LPS and IFN-γ-stimulated RAW 264.7 cells, NO production was decreased as compared to the control group. NO production from LPS/IFN-γ-activated RAW 264.7 cells was inhibited by Bn

Table 4 Anti-inflammatory activities of Bn extracts

Sample	NO generation (%)	Cell viability (%)	Sample	NO generation (%)	Cell viability (%)	Sample	NO generation (%)	Cell viability (%)
Bn-01	81.92±1.59	89.03±1.68	Bn-31	81.74±1.64	67.54±0.66	Bn-61	96.97±1.67	74.84±0.50
Bn-02	85.77±1.68	100.55±1.98	Bn-32	89.65±1.87	84.32±0.38	Bn-62	102.15±2.39	82.88±0.77
Bn-03	87.06±0.46	75.59±1.95	Bn-33	86.33±1.37	88.10±0.22	Bn-63	99.32±1.03	82.64±0.47
Bn-04	87.25±1.72	84.65±1.48	Bn-34	86.82±1.37	70.78±0.33	Bn-64	98.05±0.55	86.50±0.68
Bn-05	90.61±1.84	96.88±1.01	Bn-35	78.91±1.59	75.32±1.06	Bn-65	98.34±0.59	106.64±0.65
Bn-06	80.43±1.75	79.38±1.15	Bn-36	89.16±1.03	65.71±0.37	Bn-66	90.72±2.24	70.66±0.21
Bn-07	83.93±1.14	81.25±1.30	Bn-37	74.71±1.67	90.65±0.56	Bn-67	97.07±0.50	80.08±0.47
Bn-08	99.01±1.78	70.31±0.71	Bn-38	84.18±0.68	64.74±0.38	Bn-68	95.02±1.81	86.36±0.18
Bn-09	87.94±1.46	93.86±0.59	Bn-39	80.96±0.78	77.76±0.22	Bn-69	90.53±1.73	86.75±0.17
Bn-10	91.40±1.83	70.15±0.46	Bn-40	87.21±0.81	76.20±0.19	Bn-70	87.50±0.60	97.55±1.98
Bn-11	84.98±0.72	88.96±0.59	Bn-41	85.64±1.47	95.87±0.92	Bn-71	88.18±0.37	114.72±3.98
Bn-12	96.44±1.98	89.24±0.94	Bn-42	81.84±0.75	84.10±0.91	Bn-72	90.63±1.53	98.68±2.36
Bn-13	85.90±0.46	91.59±0.49	Bn-43	79.20±1.79	59.13±0.17	Bn-73	102.25±1.17	108.04±2.56
Bn-14	93.87±0.23	90.43±0.46	Bn-44	86.23±1.70	79.54±0.63	Bn-74	95.31±1.83	115.12±2.44
Bn-15	86.07±1.59	81.21±0.36	Bn-45	87.11±0.96	89.17±0.75	Bn-75	90.23±0.45	96.42±3.74
Bn-16	90.22±0.68	74.47±0.19	Bn-46	91.31±0.92	96.55±0.84	Bn-76	88.48±1.70	95.89±2.36
Bn-17	92.49±1.16	77.97±0.32	Bn-47	97.56±1.79	84.38±0.29	Bn-77	94.82±1.03	101.32±2.15
Bn-18	91.80±1.09	81.10±0.30	Bn-48	96.48±0.84	76.99±0.39	Bn-78	92.77±1.98	90.79±2.12
Bn-19	83.30±1.72	81.41±0.76	Bn-49	90.72±1.03	77.50±0.31	Bn-79	91.21±1.37	101.26±2.09
Bn-20	85.87±1.14	111.45±1.25	Bn-50	93.48±1.95	83.11±0.74	Bn-80	92.19±1.83	82.94±1.02
Bn-21	83.60±1.05	78.87±0.27	Bn-51	100.20±1.30	69.82±0.34	Bn-81	96.19±2.03	95.18±1.45
Bn-22	81.23±1.14	82.00±0.32	Bn-52	101.17±0.84	78.88±0.53	Bn-82	93.95±1.37	93.08±0.84
Bn-23	85.25±1.17	93.66±1.57	Bn-53	96.29±1.61	75.04±0.30	Bn-83	91.70±0.74	88.43±1.80
Bn-24	88.38±1.95	97.57±0.76	Bn-54	97.17±1.12	92.25±0.57	Bn-84	89.06±1.24	96.82±1.29
Bn-25	80.47±0.00	68.80±0.69	Bn-55	100.78±1.66	82.84±0.40	Bn-85	89.65±1.26	96.04±0.60
Bn-26	80.37±1.98	66.24±0.30	Bn-56	89.06±0.39	77.08±1.53	Bn-86	87.11±1.86	81.29±0.99
Bn-27	85.16±1.03	86.64±0.89	Bn-57	98.05±1.66	71.40±0.45	Bn-87	94.24±1.12	108.45±1.11
Bn-28	80.96±0.37	79.58±0.76	Bn-58	91.99±2.31	81.13±0.61	Bn-88	87.50±0.45	79.57±0.74
Bn-29	81.15±1.57	82.11±0.74	Bn-59	98.63±2.27	85.64±0.75	Bn-89	88.87±1.44	80.34±0.45
Bn-30	85.94±0.84	79.18±0.46	Bn-60	89.65±1.44	97.21±0.40	Bn-90	91.02±0.98	77.86±0.50
Control	100.00±1.10	85.57±3.03	Normal	33.30±0.87	100.00±1.48			
AMT*	58.03							

*AMT (10 μg/mL) was used as a positive control.

extracts (Bn-01, -11 and -37), which showed less than 85% of NO generation at a non-toxic concentration. Bn-37 significantly inhibited the production LPS/IFN-γ-induced NO at the concentration of 100 μg/mL. Therefore, Bn-37 might have the potential to be used as therapeutic agents for preventing inflammatory diseases.

Anti-cancer activity. Gastric cancer affects the gastrointestinal tract, and is the leading cause of cancer-related mortality in the world. In addition, approximately 90% of stomach cancers are adenocacinomas (Kelley and Duggan, 2003). We explored tried to explore whether Bn extracts influence the growth of AGS gastric cancer cells. To investigate the anti-cancer effect of Bn extracts, AGS cells were treated with 100 μg/mL of Bn extracts for 48 h. Cell growth was assessed by MTT assay and the result is shown in Table 5. We found that Bn-02 and -23 significantly inhibited the growth of AGS cells by greater than 85%. Bn-23 had the greatest anti-cancer effect, with growth being inhibited by 90.79%.

AR inhibition. Bn extracts were tested for their ability to inhibit rat lens AR activity, and the results are shown in Table 6. The following 11 Bn extracts (Bn-06, -07, -08, -09, -31, -33, -44, -46, -47, -48, and -52) exhibited a high degree of inhibition (>50% at 10 μg/mL) on rat lens AR. However, they were still less effective than the positive control, TMG. In a previous paper (Semwal et al., 2009), the ethanol extracts of *Boehmeria rugulosa* were studied *in vivo* for hypoglycemic effect, and showed high anti-diabetic activity. However, in our study, Bn extracts samples showed little AR activity.

In our results, Bn-13 and -82 may be rich in radical scavengers. In addition, Bn-40, -33, and -05 were most active extracts against *E. coli*, *S. aureus* and *H. pylori*, respectively. Concerning the anti-inflammatory and anti-cancer activity, Bn-37 was relatively effective on protection from LPS/IFN-γ-induced cytotoxicity and Bn-23 may contribute to the treatment or prevention of gastric cancer. On the other hand, Bn extracts were less effective on AR inhibitory activity. Consequently, the screening of Bn extracts

Table 5 Anti-cancer activities of Bn extracts

Sample	AGS cell growth inhibition (%)	Sample	AGS cell growth inhibition (%)	Sample	AGS cell growth inhibition (%)
Bn-01	46.10±0.35	Bn-31	36.87±0.62	Bn-61	26.47±0.48
Bn-02	87.99±0.14	Bn-32	32.66±0.21	Bn-62	34.33±0.16
Bn-03	26.92±0.69	Bn-33	27.50±0.18	Bn-63	20.60±0.62
Bn-04	43.08±0.30	Bn-34	26.33±0.17	Bn-64	23.10±0.40
Bn-05	28.91±0.21	Bn-35	24.06±0.57	Bn-65	21.17±0.11
Bn-06	41.51±0.55	Bn-36	39.80±0.37	Bn-66	27.53±0.50
Bn-07	50.56±0.94	Bn-37	28.14±0.34	Bn-67	28.89±0.27
Bn-08	41.30±0.25	Bn-38	46.09±0.07	Bn-68	24.52±0.80
Bn-09	29.62±0.48	Bn-39	34.09±0.41	Bn-69	21.01±0.48
Bn-10	52.21±0.43	Bn-40	37.98±1.18	Bn-70	40.26±2.31
Bn-11	32.51±0.56	Bn-41	33.15±0.48	Bn-71	22.78±3.83
Bn-12	35.72±0.54	Bn-42	26.00±3.40	Bn-72	38.13±0.30
Bn-13	28.27±0.55	Bn-43	35.86±0.83	Bn-73	37.28±1.10
Bn-14	27.79±0.10	Bn-44	35.89±0.39	Bn-74	18.83±0.31
Bn-15	35.24±0.25	Bn-45	35.28±0.26	Bn-75	37.73±0.67
Bn-16	32.51±0.24	Bn-46	36.36±0.41	Bn-76	43.82±0.15
Bn-17	41.09±0.52	Bn-47	44.96±0.58	Bn-77	45.64±0.10
Bn-18	38.85±0.51	Bn-48	46.06±0.20	Bn-78	40.13±0.45
Bn-19	35.78±1.58	Bn-49	38.84±0.20	Bn-79	40.71±0.16
Bn-20	23.50±0.11	Bn-50	33.74±0.14	Bn-80	31.28±0.17
Bn-21	44.97±0.41	Bn-51	31.77±0.61	Bn-81	36.97±0.52
Bn-22	32.94±0.50	Bn-52	29.75±0.20	Bn-82	30.79±0.25
Bn-23	90.79±0.20	Bn-53	40.81±0.31	Bn-83	25.54±0.18
Bn-24	32.98±0.37	Bn-54	28.06±0.58	Bn-84	36.41±0.14
Bn-25	25.63±4.71	Bn-55	35.49±0.87	Bn-85	37.09±2.17
Bn-26	39.73±0.55	Bn-56	44.93±0.75	Bn-86	48.77±0.26
Bn-27	32.27±0.22	Bn-57	37.31±0.15	Bn-87	40.87±1.95
Bn-28	66.13±6.14	Bn-58	30.58±0.55	Bn-88	36.11±0.79
Bn-29	33.72±1.31	Bn-59	28.02±1.21	Bn-89	19.80±0.25
Bn-30	38.94±0.66	Bn-60	34.81±0.70	Bn-90	27.82±0.15
5-FU*	58.46±0.26				

*5-FU (10 μg/mL) was used as a positive control.

Table 6 AR inhibition by Bn extracts

Sample	Inhibition (%)	Sample	Inhibition (%)	Sample	Inhibition (%)
Bn-01	18.19	Bn-31	57.49	Bn-61	14.74
Bn-02	32.28	Bn-32	-	Bn-62	30.88
Bn-03	40.53	Bn-33	60.63	Bn-63	21.75
Bn-04	10.97	Bn-34	0.00	Bn-64	22.11
Bn-05	28.50	Bn-35	0.00	Bn-65	9.47
Bn-06	57.72	Bn-36	5.58	Bn-66	-
Bn-07	53.24	Bn-37	7.67	Bn-67	17.90
Bn-08	64.94	Bn-38	0.00	Bn-68	15.44
Bn-09	56.00	Bn-39	16.03	Bn-69	17.90
Bn-10	26.12	Bn-40	37.98	Bn-70	20.35
Bn-11	20.05	Bn-41	28.75	Bn-71	26.91
Bn-12	24.25	Bn-42	-	Bn-72	10.63
Bn-13	26.55	Bn-43	-	Bn-73	31.23
Bn-14	12.78	Bn-44	52.40	Bn-74	17.94
Bn-15	17.75	Bn-45	21.09	Bn-75	18.94
Bn-16	26.55	Bn-46	54.31	Bn-76	13.95
Bn-17	17.37	Bn-47	57.51	Bn-77	21.26
Bn-18	17.76	Bn-48	63.58	Bn-78	15.95
Bn-19	20.05	Bn-49	20.13	Bn-79	18.61
Bn-20	27.33	Bn-50	25.24	Bn-80	18.94
Bn-21	18.11	Bn-51	27.74	Bn-81	25.10
Bn-22	15.28	Bn-52	50.00	Bn-82	26.24
Bn-23	19.89	Bn-53	32.32	Bn-83	15.21
Bn-24	25.20	Bn-54	23.48	Bn-84	27.00
Bn-25	14.92	Bn-55	18.90	Bn-85	33.84
Bn-26	27.69	Bn-56	40.55	Bn-86	34.98
Bn-27	8.19	Bn-57	41.77	Bn-87	25.86
Bn-28	26.98	Bn-58	48.17	Bn-88	25.48
Bn-29	3.58	Bn-59	47.56	Bn-89	30.80
Bn-30	7.13	Bn-60	40.85	Bn-90	33.46
TMG*	89.35				

*TMG was used as a positive control.

proved to be effective for the selection of those which could have biological benefits including anti-oxidant, anti-bacterial, anti-inflammatory, anti-cancer and anti-diabetic effects. However, it is necessary to carry out further studies to confirm these results.

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References

Ames BN, Shigenaga MK, and Hagen TM (1993) Oxidants, antioxidants, and the degenerative diseases of aging. *Proc Natl Acad. Sci* **90**, 7915–22.

Beutler B and Rietshcel ET (2003) Innate immune sensing and its roots: the story of endotoxin. *Nat Rev Immunol* **3**, 169–76.

Chiou WF, Sung YJ, Liao JF, Shum AY, and Chen CF (1997) Inhibitory effect of dehydroevodiamine and evodiamine on nitric oxide production in cultured murine macrophages. *J Nat Prod* **60**, 708–11.

Chung SK, Osawa T, and Kawakishi S (1997) Hydroxyl radical-scavenging effects of spices and scavengers from brown mustard (*Brassica nigra*). *Biosci Biotechnol Biochem* **61**, 118–23.

Davidson PM and Parish ME (1989) Methods for testing the efficacy of food antimicrobials. *Food Technol* **43**, 148–55.

Forman D, Newell DG, Fullerton F, Yarnell JW, Stacey AR, Wald N et al. (1991) Association between infection with *Helicobacter pylori* and risk of gastric cancer: evidence from a prospective investigation. *Br Med J* **302**, 1302–5.

Gupta K and Wagle DS (1988) Nutritional and antinutritional factors of green leafy vegetables. *J Agric Food Chem* **36**, 472–4.

Halliwell B, Gutteridge JM, and Cross CE (1992) Free radicals, antioxidants and human disease: Where are we now? *J Lab Clin Med* **119**, 598–620.

Hatano T, Edamatsu R, Hiramatsu M, Mori A, Fujita Y, Yasuhara T et al. (1989) Effects of the interaction of tannins with co-existing substances, VI. Effects of tannins and related polyphenols on superoxide anion radical and on 1,1-diphenyl-2-pricrylhydrazyl radical. *Chem Pharm Bull* **37**, 2016–21.

Kelley JR and Duggan JM (2003) Gastric cancer epidemiology and risk factors. *J Clin Epidemiol* **56**, 1–9.

Kim SI, An MJ, Han YS, and Pyeun JH (1993) Sensory and instrumental texture properties of rice cakes according to the addition of songpy (pine tree endodermis) or mosipul (China grass leaves). *J Korean Soc Food*

Nutr **22**, 603–10.
Lin CC, Yen MH, Lo TS, and Lin CF (1997) The anti-inflammatory and liver protective effects of *Boehmeria nivea* and *B. nivea* sub sp. *nippononivea* in rats. *Phytomedicine* **4**, 301–8.
Lin CC, Yen MH, Lo TS, and Lin JM (1998) Evaluation of the hepatoprotective and antioxidant activity of *Boehmeria nivea* var. *nivea* and *B. nivea* var. *tenacissima*. *J Ethnopharmacol* **60**, 9–17.
Liu F, Liang X, Zhang N, Huang Y, and Zhang S (2001) Effect of growth regulators on yield and fiber quality in ramie (*Boehmeria nivea* (L.) Gaud.), China grass. *Field Crops Res* **69**, 41–6.
Mok SY, Shin HC, and Lee S (2012) Screening of aldose reductase inhibitory activity of white-color natural products. *CNU J Agric Sci* **39**, 69–73.
Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* **65**, 55–63.
Nathan C (1992) Nitric oxide as a secretory product of mammalian cells. *FASEB J* **6**, 3051–64.
Oyaizu M (1986) Studies on products of browning reaction: antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn J Nutr Diet* **44**, 307–15.
Park SS, Kim SI, and Sim KH (2011) The quality characteristics and antioxidative activity of *Sulgidduk* supplemented with ramie leaf powder. *Kor J Food Cookery Sci* **27**, 763–72.
Rauha JP, Remes S, Heinonen M, Hopia A, Kähkönen M, Kujala T et al. (2000) Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. *Int J Food Microbiol* **56**, 3–12.
Sato S and Kador PF (1990) Inhibition of aldehyde reductase by aldose reductase inhibitors. *Biochem Pharmacol* **40**, 1033–42.
Semwal DK, Rawat U, Semwal R, Singh R, Krishan P, Singh M et al. (2009) Chemical constituents from the leaves of *Boehmeria rugulosa* with antidiabetic and antimicrobial activities. *J Asian Nat Prod Res* **11**, 1045–55.
Soares JR, Dinis TC, Cunha AP, and Almeida LM (1997) Antioxidant activities of some extracts of *Thymus zygis*. *Free Radical Res* **26**, 469–78.
Sreejayan N and Rao MN (1997) Nitric oxide scavenging by curcuminoids. *J Pharm Pharmacol* **49**, 105–7.
Wang B, Peng D, Liu L, Sun Z, Zhang N, and Gao S (2007) An efficient adventitious shoot regeneration system for ramie (*Boehmeria nivea* Gaud.) using thidiazuron. *Bot Stud* **48**, 173–80.
Wang B, Peng DX, Sun ZX, Zhang N, and Gao SM (2008) *In vitro* plant regeneration from seedling-derived explants of ramie [*Boehmeria nivea* (L.) Gaud.]. *In Vitro Cell Dev Biol-Plant* **44**, 105–11.
Zhang S, Iandolo JJ, and Stewart GC (1998) The enterotoxin D plasmid of *Stapylococcus aureus* encodes a second enterotoxin determinant (sej). *FEMS Microbiol Lett* **168**, 227–33.