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Free radical scavenging activity and kinetic behavior of the *Galgeuntang* water extract

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SUMMARY

Galgeuntang water extract exhibited a strong free radical scavenging activity and reducing power determination. However, a gradual increase in the free radical scavenging activity and reducing power determination was obtained with increasing concentrations. The highest radical scavenging activity was shown by the water extract from *Galgeuntang* (116.93 μg/mL) and the water extract from *Cinnamonum cassia* Presl. (95.01 μg/mL). These results of phenolic and flavonoid contents of the extracts indicated that the strong radical scavenging activity of the *Cinnamonum cassia* Presl. extract might be in part due to the phenolic compounds. The correlation coefficient between TPC and DPPH (r^2 = 0.9312), TFC and DPPH (r^2 = 0.9677), showed positive correlation among total phenolic/flavonoid contents and antioxidant activity. These results suggest that *Galgeuntang* has a potential antioxidant activity.

Key words: *Galgeuntang*; Free radical scavenging activity; Phenolic/flavonoid contents.

INTRODUCTION

Galgeuntang has been broadly used in traditional oriental medicine especially in China, Republic of Korea and Japan for its many profound pharmacological action. It has been applied as antipyretic and diaphoretic agents (Kinjo *et al*., 1987). *Galgeuntang* is reported to relieve hypertension, migraine, and sudden deafness by improvement of cerebral circulation (Rong *et al.,* 1998). Today, more and more people take plant medicine as an alternative therapy. *Galgeuntang* is one of the most important oriental crude drugs which increases coronary artery blood flow and is used as antidiarrhetic, antiemetic, antispasmodic and antimicrobic remedy (Tang, 1992; Zhu, 1998). *Galgeuntang* demonstrates several interesting activities including suppression of alcohol intake by alcoholics reduction of blood pressure and phytoestrogenic prevention of osteoporosis (Adam, 2004). We evaluated the antioxidant activity of *Galgeuntang* employing various assay systems, such as free radical scavenging activity, total phenolic and flavonoid content.

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MATERIALS AND METHODS

Chemicals and instruments

Octadecyl-functionalized silica gel, butylatedhydroxyanisole (BHA), β-carotene, α- tocopherol, 1,1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid,

Table 1. The composition of *Galgeuntang*

Botanical name	Weight (g)	Percentage (%)	Origin
Pueraria thunbergiana Benth.(PT)	15.00	22.06	China
Paeonia lactiflora Pall.(PL)	7.50	11.03	Republic of Korea
Ephedra sinica Fisch.(ES)	11.25	16.54	China
Glycyrrhiza uralensis Fish.(GU)	7.50	11.03	China
Zizyphus jujuba Mill.(ZJ)	8.00	11.76	Republic of Korea
Cinnamonum cassia Presl.(CC)	7.50	11.03	China
Zingiber officinale Rosc.(ZO)	11.25	16.54	Republic of Korea

ascorbic acid, sodium carbonate, ferric chloride, folin-ciocalteu reagent were purchased from Sigma-Aldrich Co. (St. Louis). Aluminum sheets (Silica gel 60 F_{254}) for TLC were from Merck, Germany. All other reagents were of analytical grade. Recordings were made in a UV–vis Diode Array Spectrophotometer, Hewlett Packard 8453. ^{1}H-NMR spectrum was rewarded on a JEOL NMR FT. 500 MHZ NMR spectrometer. Tetramethylsilane was used as an internal standard.

Plant material

Galgeuntang was obtained from Osio Herbal Clinic, Province of Kyungbuk in Republic of Korea. The voucher specimen was deposited in the Department of Herbal Resources, Professional Graduate School of Oriental Medicine, Wonkwang University in Republic of Korea (Table 1).

The water extract of *Galgeuntang*

The air-dried and powdered *Galgeuntang* were extracted with water at 80 - 85 temperature for 4 h. The extraction was done thrice and the obtained solution from *Galgeuntang* and the structural medicinal plants were collected separately and filtered using what man no 42. The solution was evaporated by using vacuum evaporator under vacuum below 75 to give the crude extracts and stored at 4 until use.

Thin-layer chromatography

Glass plates (0.25 mm), pre-coated with silica gel (Merck, Darmstadt, Germany), were spotted with of dry extract re-suspended in ethanol: water (1:1) and then developed in *n*-buthanol: acetic acid: water (4:1:5), ethyl acetate: methanol: water (77:13:10) and ethyl acetate: formic acid: water (65:15:20). The plates were then prayed with 10 g/ mL β-carotene solution, dissolved in chloroform: ethanol (1:1). The permanence of orange color after corresponds to the relative antioxidant activity of the extract. 1% potassium ferricyanide, 1% ferric chloride and 2% ferric chloride in ethanol were used to detec the presence of phenolic compounds (Duve *et al*., 1991).

DPPH radical-scavenging activity

The scavenging capacity of the water extract of *Galgeuntang* was determined, using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), as a stable free radical (Yen *et al*., 1995). For the initial screening, 0.12 mM of DPPH was used and for active extracts, higher concentration of DPPH (0.3 mM) was used. In brief, 500 uL of various concentrations of the samples in methanol was added to 500 uL of methanol solution of DPPH. The mixture was shaken vigorously and left to stand for 30 min the dark, and the absorbance was then measured at 517 nm against a blank. Extract concentration providing 50% inhibition (IC_{50}) was calculated form the graph plotting inhibition percentage against extract concentrations. Moreover, the percentage DPPH radical scavenging activity was determined using following equation.

DPPH radical scavenging (%) =

[(control absorbance-sample absorbance)/control absorbance]×100.

Determination of total phenolic content

Total phenolic content of the aforementioned water extract of *Galgeuntang* were determined by literature methods involving the folin-ciocalteu method (Iqbal and Bhanger, 2006) and gallic acid as standard. Twenty micro liters of extract solution was taken in a curve, then 1.58 ml of distilled water and 100 μl of folin-ciocalteu reagent were added, and curve was shaken thoroughly. After 3 min, 300 μl it the sodium carbonate solution (7% w/v) was added, and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm.

Determination of total flavonoid content

Total flavonoids were measured after following Zhishen *et al.* (1999). Briefly, 1 ml aliquot of appropriately diluted sample or standard solutions of rutin (20, 40, 60, 80 and 100 μg/ml) were added to a 10 ml volumetric flask containing 4 ml distilled H_2O. At zero time, 0.3 ml of 5% $NaNO_2$ was added to the flask. After 5 min, 0.3 ml of 10% $AlCl_3$ was added. At 6 min, 2 ml of 1 M NaOH was added to the mixture. Immediately, the reaction flask was diluted to volume with the addition of 2.4 ml of added to H_2O and thoroughly mixed. Absorbance of the water extract of *Galgeuntang* mixture, pink in color, was determined at 510 nm versus prepared water blank. Total flavonoids of the water extract of *Galgeuntang* were expressed on a fresh weight basis as μg/mg Rutin equivalents (CE). Samples were analyzed in five replications. The calibration curve range of rutin was 20-300 μg/ml (r^2 = 0.9831).

Statistical analysis

The data are results of triplicate experiments. Microsoft Excel was used to compute means, standard deviation, correlation and regression. Differences among all sample means were determined by analysis of variance (ANOVA) using Origin (Micro cal Software, Inc.) and were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

DPPH radical-scavenging activity

The yields and free radical scavenging activity of the water exact of *Galgeuntang* are given in Table 2. Yields of the water extract ranged from 2.05% (*Zingiber officinale* Rosc.) to 25.33% (*Pueraria thunbergiana* Benth.). The antiradical potential of the *Galgeuntang* extract was determined by using very stable free radicals 1,1-diphenyl-2-picrylhydrazyl (DPPH). *Galgeuntang* extract showed a range of radical scavenging activity. The IC_{50} value was in

Table 2. Yield and free radical scavenging activity of the *Galgeuntang* water extracts

Tested material	IC_{50} (μg/mL)	Yield (%)
Pueraria thunbergiana Benth.	171.18 ± 0.23	25.33
Paeonia lactiflora Pall.	136.84 ± 0.61	3.38
Ephedra sinica Fisch.	211.60 ± 1.01	11.88
Glycyrrhiza uralensis Fisch.	1752.65 ± 12.21	22.09
Zizyphus jujuba Mill.	1238.41 ± 18.83	20.05
Zingiber officinale Rosc.	663.47 ± 7.41	2.05
Cinnamonum cassia Presl.	95.01 ± 0.10*	2.36
Galgeuntang	116.93 ± 0.36	13.02
BHA	85.14 ± 0.23**	

The values are expressed as mean ± S.D. (n = 3). BHA: Butylhydroxyanisole, Significantly different from the control values. *$P < 0.05$, **$P < 0.01$.

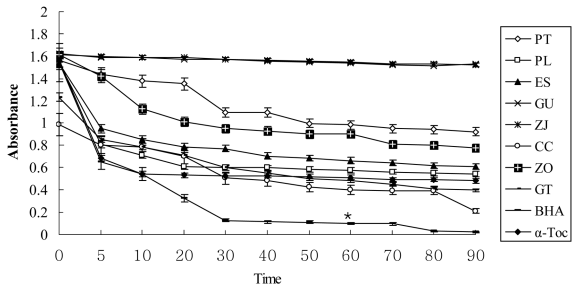

Fig. 1. Antioxidative kinetics of the *Galgeuntang* water extracts against DPPH method at 120 μg/ml concentration. The values are expressed as mean ± S.D. (n = 3). Significantly different from the control values. $^{*}P < 0.05$. PT: Water extract of *Pueraria thunbergiana* Benth; PL: Water extract of *Paeonia lactiflora* Pall. ES: Water extract of *Ephedra sinica* Fisch. GU: Water extract of *Glycyrrhiza uralensis* Fisch. ZJ: Water extract of *Zizyphus jujuba* Mill. CC: Water extract of *Cinnamonum cassia* Presl. ZO: Water extract of *Zingiber officinale* Rosc. GT: *Galgeuntang;* BHA: butylhydroxyanisole; α-Toc: α-Tocopherol.

between 95.01 μg/ml to 1,752.65 μg/ml. The strongest radical scavenging activity was shown by stem extract of *Cinnamonum cassia* Presl (Table 2). Radical scavenging activities are important due to the deleterious role of free radicals in foods and in biological systems (Yokozawa *et al*., 1998). DPPH assay evaluates the ability of antioxidants to scavenge free radicals. The method is based on the reduction of alcoholic DPPH solution into non-radical form DPPH-H in the presence of a hydrogen-donating antioxidant (Bouchet *et al*., 1998). During initial screening low absorbance was observed for active extracts even in low concentration of sample. Hence, higher concentration of DPPH (0.3 mM) was used and results were compared with reference compounds. As illustrated in, all extracts exhibited a concentration-dependent DPPH radical scavenging activity (Cai *et al*., 2004). The free radical scavenging activity of all the extracts was lower than that of natural antioxidant vitamin E at all tested concentrations, showing the presence of the strong radical scavenging compounds. *Galgeuntang* (GT) showed a strong DPPH radical-scavenging activity. The radical scavenging activity of the water extracts and reference compounds was in following order; BHA > CC > GT > PL > PT > ES > ZO > PB > ZJ > GU (Table 2). Many phenolic compounds have been reported to possess potent antioxidant activity, which vary according to the number and position of hydroxyl groups. Comparisons among the different classes of phenolic compounds showed that tannins were the most potential towards DPPH radical scavenging (Bouchet *et al*., 1998; Yokozawa *et al*., 1998; Cai *et al*., 2004). All of these plants contain a number of phenolic compounds including tannins (Kadota *et al*., 1990; Surveswaran *et al*., 2007). Hence, the strong DPPH radical scavenging potency of the extracts might be attributed towards these compounds. For the detection of antioxidant compounds from the ethanol extracts. TLC analysis was carried out. The TLC plates of the extracts developed in all three elution systems showed remaining orange spots until 12 h after spraying with a â-carotene solution, which indicated the presence of compounds with strong antioxidant ability.

Present study showed that *Galgeuntang* extract displayed highly potent antioxidant activity. From the results shown in (Table 3), the commercial antioxidant BHA and β-tocopherol was used as an antioxidant standard. The radical scavenging activity of the tested samples, expressed as inhibition percentage IP (%), is given in Tables 3. The (IP %) was calculated by the following formula: IP (%) = $[(A_B - A_A) / A_B] \times 100$, where A_B is

Table 3. Free radical scavenging activity (IP %) against DPPH (0.3 mM) of the *Galgeuntang* water extract at 30 min

Conc.	*Galgeuntang* extract								α-Toco	BHA
(μg/ml)	20	40	80	100	120	140	180	200	20	20
IP (%)	2.20	39.00	34.00	54.00	61.50	42.00	42.70	43.21	48.54	78.31

BHA: Butylhydroxyanisole; α-Toc: α-Tocopherol.

the absorbance of the blank sample (t = 0), and A_A is × 100, where A_B is the absorbance of the blank sample (t = 0), and A_A is the absorbance of the tested sample after 30 min (Joaquin *et al*., 2005). The results of radical scavenging activity were summarized in Table 3 and had effective free radical scavenging activities at a concentration of 120 μg/ml (IP = 61.50%).

The antioxidative kinetics, according to the time at the steady state, have been reported as rapid at less than 5 min, and intermediate from 5 to 90 min. In this study, the reaction rate of the *Galgeuntang* water extract and BHA was slow while that of α-tocopherol was intermediate, which agreed with previous studies showing that the antioxidative kinetic classification of BHA was slow and of α-tocopherol was intermediate (Fig. 1). This clearly showed that in spite of its slow reaction rate, the *Galgeuntang* water extract could scavenge the free radicals.

Analysis of total phenolics and flavonoid contents of the *Galgeuntang* water extract

The antioxidant activity and radical-scavenging ability of *Galgeuntang* extract attributed to the antioxidant compounds including phenolics, flavonoid, ascorbic acid, α-tocopherol and carotenoids, etc. Thus, the total phenolic and flavonoid contents of the extract were evaluated. The total phenolic content was determined using folin-ciocalteu ethod and results were expressed as gallic acid equivalents (GAE). From the results shown in Table 4, the content of total phenolic of *Cinnamonum cassia* Presl. extract is the highest among all of the samples, followed by the other water extract of *Galgeuntang*. Similarly, the total flavonoid content (TFC) was determined following Zhishen *et al.* (1999) and results were expressed as rutin equivalents. Like TPC, the TFC of *Cinnamonum cassia* Presl. extract was higher than that of the other seven extracts of *Galgeuntang*. The results indicated that the strong antioxidant and radical scavenging activity of the *Cinnamonum cassia* Presl. extract might be in part due to the phenolic compounds. The correlation coefficient between TPC and DPPH (r^2 = 0.9312), TFC and DPPH (r^2 = 0.9677), and TFC (r^2 = 0.80) showed positive correlation among total phenolic/flavonoid contents and antioxidant activity.

Analytical data of nuclear magnetic resonance spectrometry

We standardized the water extract from *Galgeungtang* by nuclear magnetic resonance spectrometry (Varian Unity 500, 500 MHz, Japan). The ethyl acetate extract was analyzed by ^{1}H-NMR spectroscopy of DMSO-d_6 soluble. It gave the main biological compound, which the signals were clearly visible in the ^{1}H-NMR spectrum. Perhaps, the obvious were chemical shifts were between weak 3.0 ppm

Table 4. Analysis of total phenolics/flavonoid contents of the *Galgeuntang* water extracts

Tested material	IC_{50} (μg/mL)	
	TPC	TFC
Pueraria thunbergiana Benth.	92.43 ± 0.46	7.36 ± 0.21
Paeonia lactiflora Pall.	115.19 ± 0.93*	9.25 ± 0.15*
Ephedra sinica Fisch.	116.85 ± 0.83	9.03 ± 0.46
Glycyrrhiza uralensis Fisch.	15.38 ± 1.16	2.13 ± 0.37
Zizyphus jujuba Mill.	41.39 ± 0.86	3.14 ± 0.40
Cinnamonum cassia Presl.	137.74 ± 0.53**	12.88 ± 0.58**
Zingiber officinale Rosc.	100.99 ± 1.08	8.26 ± 0.55
Galgeuntang	121.33 ± 0.94	10.35 ± 0.40

The values are expressed as mean ± S.D. (n = 3). Significantly different from the control values. *P < 0.05, **P < 0.01.

and 4.0 ppm indicative of a glycogen of glucosides group, C-ring of isoflavonoid groups between 4.8 ppm and 5.2 ppm, and A-ring of isoflavonoid group between 4.8 ppm and 5.2 ppm, and A-ring of isoflavonoid group between 7.8 ppm and 8.3 ppm in isoflavonoid - type compounds (Lee *et al*., 1994).

In conclusion, the already strong free radical scavenging activity of the traditional herbal oriental medicine, *Galgeuntang* water extract, was further increased with increasing concentrations. *Galgeuntang* was evaluated by free radical scavenging assay using 1,1-diphenyl-2-picrylhydrazyl (DPPH). The highest radical scavenging activity was shown by the *Galgeuntang* water extract (116.93 μg/ml), followed by the water extract from *Cinnamomum cassia* Presl. (95.01 μg/ml). The *Galgeuntang* water extract showed the highest inhibition percentage (IP) value at 120 μg/ml (61.50%). The investigation into the phenolic and flavonoid contents of the extracts indicated that the strong radical scavenging activity of the *Cinnamomum cassia* Presl. extract might be partially due to the phenolic compounds. The correlation coefficients between total phenolic content (TPC) and DPPH (r^2 = 0.9312) demonstrated the positive correlation among antioxidant activity. The *Galgeuntang* water extract plays a prominent role in the potential antioxidative ability.

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