Prev Nutr Food Sci
Vol 17, p 306~309 (2012)
http://dx.doi.org/10.3746/pnf.2012.17.4.306

Anti-oxidant Activity of *Saussurea lappa* C.B. Clarke Roots

− Research Note −

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Abstract

This study was performed to investigate the potential use of *Saussurea lappa* C.B. Clarke as a source of anti-oxidant agents. Various solvent fractionates from *S. lappa* C.B. Clarke roots were investigated for their anti-oxidative effectiveness. The contents of total phenolics and flavonoids were determined by the Folin-Ciocalteu's colorimetric and the aluminum nitrate method, respectively. Total phenolic and flavonoid contents of *n*-butanol soluble fractionates from *S. lappa* C.B. Clarke, 44.43 μg gallic acid equilibrium (GAE)/g extract and 92.15 μg quercetin equilibrium (QE)/g extract, respectively, were higher than those of other solvent fractionates. The *n*-butanol soluble fractionates of *S. lappa* C.B. Clarke (1,000 ppm) showed the strongest inhibitory potential on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and reducing power at 92.98% and 0.38, respectively. Thus, our data shows that the *S. lappa* C.B. Clarke plant may help prevent antioxidative stress.

Key words: *Saussurea lappa* C.B. Clarke, antioxidant activity, quercetin equilibrium, 2,2-diphenyl-1-picrylhydrazyl, scavenging activity

INTRODUCTION

Saussurea lappa C.B. Clarke, belonging to the family *Asteraceae* and one of the best-known species within its genus, is a perennial, aromatic, and medicinal plant (1). *S. lappa* C.B. Clarke has been used in folk medicine for the treatment of various ailments and diseases such as viz., asthma, certain bronchitis, ulcer, and stomach problems since ancient times (2-4); furthermore, according to previous findings, the plant inhibits the growth, acid production, adhesion, and water-insoluble glucan synthesis of *Streptococcus mutans*. Anti-*Helicobacter pylori* action to treat ulcer diseases, and therapeutic effects, such as halitosis, dental caries, and periodontal diseases of *S. lappa* C.B. Clarke have also been investigated (5,6); moreover, several reports indicate that the plant has hepatoprotective, anti-parasitic, CNS depressant, anti-ulcer, immunomodulator, and anti-cancer abilities (7, 8). Some naturally occurring compounds found in edible and medicinal plants, herbs, and spices have also been well known to possess the antioxidative and antimicrobial activities against food-borne pathogenic bacteria (9-11); in particular, flavonoid-rich plant extracts have been reported to exhibit antibacterial and antioxidative activities (12). *Saussurea lappa* C.B. Clarke includes lactones, such as costunolide, 13-methoxy-11,13-dihydrodehydrocostuslactone, dehydrocostus lactone, and aldehyde-sesquiterpene lactones 4 and 5. Sesquiterpene lactones, including costunolide and dehydrocostus lactone, are major components of the roots (2), and have been suggested to possess various biological activities, such as antiviral, antifungal, anti-tumor, anti-ulcer, anti-inflammatory, neurocytotoxic and cardiotonic activities (8). Although, few studies have investigated the antioxidant effect of *S. lappa* C.B. Clarke root extracts, we investigated the anti-oxidative effectiveness of the plant roots using various solvent fractionates in this study.

MATERIALS AND METHODS

Preparation of ethanol extracts and solvent fractionates

Saussurea lappa C.B. Clarke was purchased from Kyung-dong herbal market (Seoul, Korea) in 2006. Dried and ground *S. lappa* C.B. Clarke roots were extracted with ethanol at room temperature, and then supernatant was filtered and evaporated in a vacuum below 50°C using a rotary evaporator (EYELA, Tokyo, Japan). The extracts of *S. lappa* C.B. Clarke were fractionated successively with hexane, chloroform, and *n*-butanol. These fractionates were concentrated by evaporation or drying. Each fractionate was dissolved in media and then filtered through a syringe filter (0.45 μm). Extracts and solvent fractionates were used to test antioxidant activity.

Determination of total phenolic and flavonoid contents

The total phenolic content was measured by a colorimetric assay described previously (13). Gallic acid was used to calculate the standard curve (0.01~0.1 mM). Estimation of the phenolic compounds were carried out

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in triplicate. The results were mean values ± standard deviations (SD) and expressed as mg of gallic acid equivalents/g of extract (GAEs). Total flavonoid contents were measured according to the method devised by Choi et al. (14), and calculated as quercetin equivalents using calibration curves prepared with quercetin standard solutions covering a concentration range between 0 and 0.05 mg/mL. The results were expressed as mean values ± SD, after triplicate analysis. The data were calculated as GAEs and quercetin equivalents/g-extract (QEs) for total phenolic and flavonoid contents, respectively. The results performed in triplicate were expressed as mean values ± SD.

Determination of antioxidant activity

2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity: Scavenging effects of *S. lappa* C.B. Clarke (100 ~ 1,000 ppm) on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals were monitored according to the method described by Lee et al. (15). A 0.2 mL of methanolic solution containing extracts was mixed with 4 mL of methanol, and a methanol tion of DPPH (1 mM, 0.5 mL) was added. The mixture was placed onto the vortex for 15 sec, left to stand at room temperature for 30 min, and the absorbance read at 517 nm.

Reducing power: The reducing power of *S. lappa* C.B. Clarke was determined by Fe^{3+} reduction (16). The solvent fractionates (100 ~ 1,000 ppm) in distilled water were mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% $K_3Fe(CN)_6$. The mixture was incubated at 50°C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid was added and centrifuged at 2,000 × *g* for 10 min. A 2.5 mL of the supernatant layer was added to 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$. The absorbance of the mixture was measured at 700 nm using UV-spectrophotometry (Agilent Technologies Inc., Santa Clara, CA, USA).

RESULTS AND DISCUSSION

Total phenolic and flavonoid contents

The total phenolic and flavonoid contents of ethanol extracts (ex.) and solvent fractionates (fr.) from *S. lappa* C.B. Clarke are shown in Table 1. The *S. lappa* C.B. Clarke samples tested were: ethanol ex. (SRI), hexane fr. (SRII), *n*-chloroform fr. (SRIII), and *n*-butanol fr. (SRIV).

The results were mean values ± standard deviations (SD) of duplicate analyses of three replications. The results of the total phenolic contents (μg GAE/g extract) of the samples trended in the following manner: *n*-butanol fr. (44.54 ± 0.61 μg) > chloroform fr. (33.79 ± 0.12 μg) > ethanol ex. (27.52 ± 0.07 μg) > *n*-hexane fr. (20.36 ± 0.14 μg). In addition, total flavonoid contents in each extract (μg QE/g extract) and solvent fractionate resulted in the following: *n*-butanol fr. (92.15 ± 0.05 μg) > chloroform fr. (68.56 ± 0.08 μg) > ethanol ex. (50.75 ± 0.11 μg) > *n*-hexane fr. (15.72 ± 0.13 μg). Total phenolic and flavonoid contents of *n*-butanol soluble fr. from *S. lappa* C.B. Clarke roots were higher than other solvent fr. and the ethanol extract.

Table 1. Total phenolic and flavonoid contents of *S. lappa* C.B. Clarke roots

Samples[1)]	Total phenolic content (μg GAE/g extract)	Total flavonoid content (μg QE/g extract)
SRI	27.52 ± 0.07	50.75 ± 0.11
SRII	20.36 ± 0.14	15.72 ± 0.13
SRIII	33.79 ± 0.12	68.56 ± 0.08
SRIV	44.54 ± 0.61	92.15 ± 0.05

[1)]SRI is *S. lappa* C.B. Clarke ethanol ex., SRII is *S. lappa* C.B. Clarke hexane fr., SRIII is *S. lappa* C.B. Clarke chloroform fr., SRIV is *S. lappa* C.B. Clarke *n*-butanol fr.
The results were mean values ± standard deviations (SD) of duplicate analysis of three replications.

Anti-oxidant activity

DPPH is a stable free radical that accepts an electron or hydrogen radical and becomes a stable diamagnetic molecule (17). Table 2 shows the DPPH radical-scavenging ability of the ethanol extract and solvent fractionates from *S. lappa* C.B. Clarke roots, the same testing samples used previously for total phenolic and flavonoid contents. The results were mean values ± standard deviations (SD) of duplicate analyses of three replications.

The resulting ability of each sample to scavenge DPPH radicals of *S. lappa* C.B. Clarke was in the following order: *n*-butanol fr. (95.71 ± 0.36%) > chloroform fr. (85.60 ± 1.61%) > ethanol ex. (76.04 ± 4.91%) > *n*-hexane fr. (15.42 ± 0.82) at a 500 ppm concentration; the *n*-butanol fraction showed the strongest activity. At a concentration of 1000 ppm, the ethanol extract and *n*-butanol fraction showed the strongest activities, 95.02 and 92.98 %, respectively, comparably identical to that of the ascorbic acid positive control. Reducing power of *S. lappa* C.B. Clarke ethanol extract and solvent fractionates are also shown in Table 2. Activity of the reducing power is generally associated with the presence of reductones, which have been shown to exert an antioxidant effect by donating a hydrogen atom and breaking the free radical chain (17). The reducing power for each ethanol extract and solvent fractionates of *S. lappa* C.B. Clarke was assessed with different concentrations. The ability of reducing power from *S. lappa* C.B. Clarke samples was as follows: *n*-butanol fr. (0.11 ± 0.02) > ethanol ex. (0.10 ± 0.01) > chloroform fr. (0.09 ± 0.01) > *n*-hexane fr. 0.07 ± 0.03) at the concentration of 500 ppm;

Table 2. Antioxidant activity of the ethanol extract and solvent fractionates from *S. lappa* C.B. Clarke roots

Samples[1)]	Antioxidant activity					
	DPPH radical scavenge (% control)			Reducing power (O.D.$_{700}$)		
	100 ppm	500 ppm	1,000 ppm	100 ppm	500 ppm	1,000 ppm
SRI	15.18±10.32[2)]	76.04±4.91	95.02±0.71	0.06±0.02	0.10±0.01	0.09±0.01
SRII	3.80±1.48	15.42±0.82	27.64±0.41	0.02±0.00	0.07±0.03	0.12±0.02
SRIII	31.90±11.30	85.60±1.61	85.00±4.34	0.06±0.00	0.09±0.01	0.08±0.01
SRIV	64.05±20.63	95.71±0.36	92.98±0.55	0.07±0.00	0.11±0.02	0.38±0.15
P.C.	100±0.00[3)]	100±0.00[3)]	100±0.00[3)]	1.89±0.15[4)]	2.22±0.04[4)]	2.18±0.00[4)]

[1)]SRI is *S. lappa* C.B. Clarke ethanol ex., SRII is *S. lappa* C.B. Clarke hexane fr., SRIII is *S. lappa* C.B. Clarke chloroform fr., SRIV is *S. lappa* C.B. Clarke *n*-butanol fr., P.C. is positive control.
[2)]Values are means±SD.
[3)]Ascorbic acid was used as a positive control for the DPPH test.
[4)]Pyrogallol was used as a positive control for the reducing power test. Reducing power of water (control) was 0.05±0.01 at O.D.700 nm.

the *n*-butanol fraction and ethanol extract showed the greatest reducing power. At the concentration of 1000 ppm, the *n*-butanol fraction of *S. lappa* C.B. Clarke showed the strongest DPPH radical scavenging activity (92.98%) and reducing power (0.38), a level comparably identical to that of water (control, 0.05±0.01). Anti-oxidative activities of *S. lappa* C.B. Clarke on both test systems increased in a concentration-dependent manner. According to the DPPH radical scavenging experiment, when the concentration of chloroform fr. increased from 500 to 1000 ppm, the activity decreased from 85.60% to 85.00%. A similar trend also occurred with *n*-butanol fr., whereby the radical scavenging activities were 95.71% and 92.82% for 500 and 1000 ppm concentrations, respectively. Our study on *S. lappa* C.B. Clarke revealed cell toxicity at even higher concentrations for some fractions; thus, future experiments will assess cell toxicity of *S. lappa* C.B. Clarke. We expect that the root of *S. lappa* C.B. Clarke can be used as an alternative antioxidant agent in the medical and food industry provided that the toxicity associated with high concentrations be resolved in future study.

ACKNOWLEDGMENTS

This work was supported by Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012-041653).

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(Received April 19, 2012; Accepted October 29, 2012)