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

- Research Note -

Kyung-Mi Chang[1], Soo-Im Choi[1], Sophia J. Chung[2], and Gun-Hee Kim[1†]

[1]*Plant Resources Research Institute, Duksung Women's University, Seoul 132-714, Korea*
[2]*College of Nursing, The University of Iowa, IA 52242-1121, USA*

Abstract

We investigated the total phenolic and flavonoid contents and the antimicrobial activity of ethanol extracts obtained from *Saussurea lappa* C.B. Clarke. The ethanol extracts of *S. lappa* C.B. Clarke were fractionated with various solvents (*n*-hexane, chloroform, and *n*-butanol). The antimicrobial activity of *S. lappa* C.B. Clarke was examined by disc-diffusion and micro-dilution susceptibility assays with six food-borne pathogens, and compared to that of the synthetic antibiotics. It is found that the *S. lappa* C.B. Clarke ethanol extract and *n*-hexane fraction have strong activity against *B. cereus* and *V. parahaemolyticus* strains compared to ampicillin. The inhibitory concentration (IC_{50}) values of hexane fraction against *L. monocytogenes*, *B. cereus*, and *B. subtilis* were 62.5, 250 and 500 ppm, respectively. Therefore, these data suggest that *S. lappa* C.B. Clarke may be useful as antimicrobial agents against food-borne pathogens.

Key words: *Saussurea lappa* C.B. Clarke, antimicrobial activity, disc-diffusion assay, micro-dilution susceptibility assay, minimum inhibitory concentration

INTRODUCTION

Because of increasing concerns over food safety and the potential effects of synthetic additives on health, interest in biological activities of phytochemicals, including antimicrobial activity has increased significantly (1). *Saussurea lappa* C.B. Clarke belongs to the family *Asteraceae*, one of the best-known species within this genus, and which is a perennial, aromatic, and medicinal plant (2). This plant has been used since ancient times as a folk medicine for the treatment of various ailments and diseases such as asthma, certain forms of bronchitis, ulcers, and stomach problems (3,4). Reports indicate this plant inhibits the growth, acid production, adhesion, and water-insoluble glucan synthesis of *Streptococcus mutans* (5). Its anti-*Helicobacter pylori* action to treat ulcer diseases, and therapeutic effects such as halitosis, dental caries, and periodonatal diseases have also been investigated (6). Moreover, it has shown to have hepato-protective abilities and anti parasitic, CNS depressant, and anti-ulcer, immunomodulator, and anti-cancer ability (7,8). Flavonoid-rich plant extracts have been reported to exhibit antibacterial and anti-oxidative activities (9-11). However, a few studies have investigated the antimicrobial effect for extracts of *Saussurea lappa* C.B. Clarke against foodborne pathogenic bacteria. In the present study, we investigated the antimicrobial activity of the various solvent fractions from *S. lappa* C.B. Clarke, an aromatic medicinal plant.

MATERIALS AND METHODS

Preparation of ethanol extracts and solvent fractionates

S. lappa C.B. Clarke was purchased from Gyungdong Herbal Market (Seoul, Korea) in 2006, and was authenticated by Professor Choon-Sik Chung, College of Pharmacy, Duksung Women's University. *S. lappa* C.B. Clarke roots were deposited in the Plant Resources Research Institute, Duksung Women's University, Korea. Dried and ground *S. lappa* C.B. Clarke roots were extracted with ethanol at room temperature, and the supernatant was filtered and evaporated in a vacuum below 50°C using a rotary evaporator (EYELA, Tokyo, Japan). The ethanol extracts of *S. lappa* C.B. Clarke were fractionated successively with *n*-hexane, chloroform, and *n*-butanol. These fractions were concentrated by evaporation or dryness. Each fraction was dissolved in media and then filtered using a syringe filter (0.45 μm). Extracts and solvent fractions were used as a sample to test antimicrobial activity.

Determination of total phenolic and flavonoid content

Total phenolic content was measured using a previously described colorimetric assay (12). Gallic acid was used to calculate the standard curve (0.01~0.1 mM). Estimation of the phenolic compounds was carried out in triplicate. The results were mean values±standard devi-

†Corresponding author. E-mail: ghkim@duksung.ac.kr
Phone: +82-2-901-8496, Fax: +82-2-901-8474

ations (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. (13), and calculated as quercetin equivalents using calibration curves prepared with quercetin standard solutions covering a concentration range between 0 and 0.05 mg/mL. Estimation of the flavonoid compounds was carried out in triplicate. The results were mean values ±SD and expressed as mg of quercetin equivalents/g of extract (QEs). Statistical significance was determined by Tukey test.

Determination of antimicrobial activity

The antimicrobial activity of *S. lappa* C.B. Clarke roots was examined using the disc-diffusion and micro-dilution susceptibility assays with six foodborne pathogens: the gram-positive bacteria *L. monocytogenes*, *B. cereus*, *B. subtilis*, and *S. aureus* and the gram-negative bacteria *S. choleraesuis* and *V. parahaemolyticus*. Also, antibacterial properties of *S. lappa* C.B. Clarke roots were compared to the synthetic antibiotics ampicillin and tetracycline.

Disc-diffusion method

The disc-diffusion method was performed using cotton swabs for each bacterial suspension (0.1, O.D. 600 nm) and inoculating in plates where the bacteria were spread uniformly on the agar surface. Each extract and solvent fraction was dissolved in DMSO to a final concentration of 100 mg/mL. There was no inhibitory effect for the tested microorganism growth at the final concentration of 10% DMSO and 5% ethanol. The 8 mm diameter discs were impregnated with 10 μL essential oils/disc or 200 μg extracts/disc and placed on the inoculated agar. The plates were incubated at 37°C for 12 hr and examined to verify the inhibition. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. Each assay in this experiment was repeated in triplicate, and expressed mean values±SD.

Micro-dilution susceptibility assay

The minimum inhibition concentration was measured using a turbidity assay. All pathogens tested (0.1, O.D. 600 nm) were inoculated into 2 mL of NB media along with test extracts (62.5～500 ppm) and antibiotics (6.25～100 ppm), and then incubated at 37°C for 24 hr. Antibiotics (ampicillin and tetracycline) were used as positive controls, along with appropriate vehicle controls for each extract or fraction. After incubation, the O.D. was measured at 600 nm. To determine the minimum inhibition concentration, 50 μL of each culture broth was taken from each tube, spread over sterile nutrient agar plates, and incubated at 37°C for 24 hr. Each assay in this experiment was repeated in triplicate.

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

	Total phenolic content (μg GAE/g extract)	Total flavonoid content (μg QE/g extract)
Ethanol Ex.	27.52±0.072[1)]	50.75±0.11
n-Hexane Fr.	20.36±0.14	15.72±0.13
Chloroform Fr.	33.79±0.12	68.56±0.08
n-Butanol Fr.	44.54±0.61	92.15±0.05

[1)]Mean±SD.

RESULTS AND DISCUSSION

Total phenolic and flavonoid contents

The total phenolic and flavonoid contents of ethanol extracts and solvent fractions of *S. lappa* C.B. Clarke are shown in Table 1. The levels of total phenolic content (μg GAE/g extract) in each sample were as follows: *n*-butanol fraction (44.54±0.61 μg) > chloroform fraction (33.79±0.12 μg) > ethanol extracts (27.52±0.07 μg) > *n*-hexane fraction (20.36±0.14 μg) (F 3067.783, $p<0.01$). In addition, the total flavonoid content levels (μg QE/g extract) were *n*-butanol fraction (92.15±0.05 μg) > chloroform fraction (68.56±0.08 μg) > ethanol extracts (50.75±0.11 μg) > *n*-hexane fraction (15.72±0.13 μg) (F 328079.712, $p<0.01$). The highest total phenolic and flavonoids content was observed in the *n*-butanol fraction.

Antimicrobial activity

The antimicrobial activity of *S. lappa* C.B. Clarke roots was examined using the disc-diffusion and micro-dilution susceptibility assays with six foodborne pathogens (*L. monocytogenes*, *B. cereus*, *B. subtilis*, *S. aureus*, *S. choleraesuis*, and *V. parahaemolyticus*). Also, the antibacterial property of *S. lappa* C.B. Clarke roots was compared to the synthetic antibiotics ampicillin and tetracycline. The results obtained in the evaluation of the antimicrobial activity by using an agar diffusion assay are shown in Table 2. Among the tested samples, *S. lappa* C.B. Clarke ethanol extracts and *n*-hexane fraction (each 200 μg/disc) showed growth inhibitory activity in the majority of the pathogens tested, including *L. monocytogenes*, *B. cereus*, *B. subtilis*, and *V. parahaemolyticus*. At a concentration of 200 μg/mL, *S. lappa* C.B. Clarke *n*-hexane fraction showed the strongest antimicrobial activity against *V. parahaemolyticus* strains (14±0.5) compared with the antibiotic ampicillin. *V. parahaemolyticus*, *B. subtilis* was inhibited by the treatment of ethanol extracts (11±0.2 mm) and *n*-hexane fraction (13±0.3 mm), and was compared to the syn-

Table 2. Antimicrobial activity of *S. lappa* C.B. Clarke roots

Tested pathogens	*S. lappa* C.B. Clarke Roots					Antibiotics (12.5 μg/disc)	
	Concentration (μg/mL)	Ethanol Ex.	*n*-Hexane Fr.	Chloroform Fr.	*n*-Butanol Fr.	Ampicillin	Tetracycline
L. monocytogenes	200	11±0.2[1)]	14±0.5	12±0.5	12±0.5	15±0.3	23±0.5
	100	11±0.9	10±0.3	11±0.9	10±0.3		
	50	−[2)]	−	−	10±0.7		
B. cereus	200	11±0.9	11±0.2	10±0.3	−	−	16±0.3
	100	−	10±0.7	−	−		
	50	−	−	−	−		
B. subtilis	200	11±0.2	13±0.3	−	−	12±0.3	19±0.3
	100	−	11±0.6	−	−		
	50	−	−	−	−		
S. choleraesuis	200	10±0.3	−	11±0.9	−	23±0.5	14±0.5
	100	−	−	10±0.3	−		
	50	−	−	10±0.7	−		
S. aureus	200	−	13±0.3	12±0.3	−	13±0.5	17±0.3
	100	−	−	−	−		
	50	−	−	−	−		
V. parahaemolyticus	200	16±0.1	23±0.4	14±0.8	−	−	17±0.3
	100	11±0.2	14±0.5	11±0.9	−		
	50	10±0.3	11±0.6	10±1.0	−		

[1)]Zone of inhibition (mean±SD). [2)]No inhibition.

thetic antibiotics. Lee et al. (14), have reported that *Schisandrae* fructus exhibited antibacterial activity against *Salmonella* strains of three different serotypes. As shown in Table 2, at the concentration of 200 μg/mL, the *n*-hexane fraction showed strong antimicrobial activity against *V. parahaemolyticus* strains (14±0.5) compared with the antibiotic ampicillin. The growth of *V. parahaemolyticus* was significantly inhibited by the presence of the ethanol extracts, the *n*-hexane fraction, and the chloroform fraction, with inhibition zones of 16±0.1, 23±0.4 and 14±0.8 mm, respectively. *V. parahaemolyticus* is a human pathogen (a halophilic bacterium) that occurs naturally and is widely distributed in marine environments. It is frequently isolated from a variety of raw sea foods including codfish, sardine, mackerel, lobster, scallop and oyster (15). Particularly, *V. parahaemolyticus* is characterized as more acid tolerant than most food-borne pathogens and is recognized as an important seafood pathogen (16). Lin et al. (17), reported that *V. parahaemolyticus* could survive when lactic acid was used as the only antimicrobial agent at pH 6.0, but the sensitivity of *V. parahaemolyticus* to lactic acid increased in a combination with phenolic phytochemicals which may create a low pH microenvironment themselves and cell membrane disruption due to stacking. *n*-Butanol sensitivity was examined against *L. monocytogenes*, *S. choleraesuis*, and *V. parahaemolyticus*. *n*-Butanol displayed sensitivity only against *L. monocytogenes*. The *n*-hexane fraction showed the most effective antibacterial activities against tested pathogens. Further, the *n*-hexane fraction of *S. lappa* C.B. Clarke that presented the strongest inhibition zone in the agar diffusion method was added to NB media to determine the growth inhibitory activity against these pathogens by using micro-dilution susceptibility assays. The results showed that *n*-butanol strongly inhibited the growth of all of the tested pathogens in a dose-dependent manner (Fig. 1). As shown Fig. 1, the MIC of the *n*-hexane fraction against *V. parahaemolyticus* was quite similar to that of tetracycline at 500 ppm. It was observed that the inhibitory concentration (IC_{50}) values of the *n*-hexane fraction against *L. monocytogenes*, *B. cereus,* and *B. subtilis* were 62.5, 250 and 500 ppm, respectively. Ethanol extracts and the *n*-hexane fraction of *S. lappa* C.B. Clarke showed strong antibacterial activity against *V. parahaemolyticus* strains. It is possible to conclude that *S. lappa* C.B. Clarke has antimicrobial potential activity against food-borne bacteria and can likely be used as an alternative phytochemical preservative in the food safety industry.

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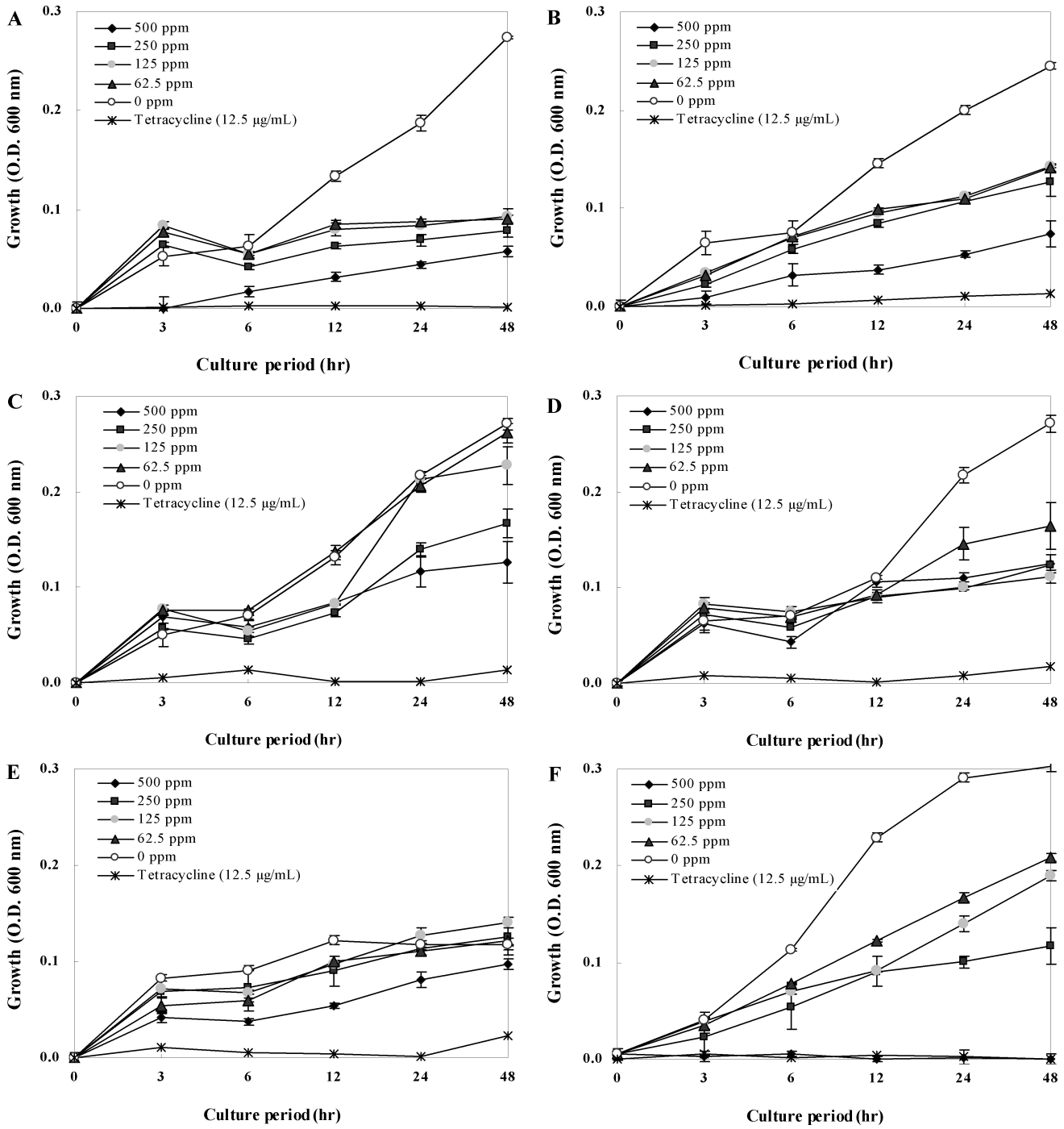

Fig. 1. Foodborne pathogens growth kinetics of *S. lappa* C.B. Clarke. A: *L. monocytogenes*, B: *B. cereus*, C: *B. subtilis*, D: *S. choleraesuis*, E: *S. aureus*, F: *V. parahaemolyticus*.

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