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Antimicrobial Effect of *Sophora angustifolia* Extracts on Food-Borne Pathogens

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Abstract This study was performed to investigate the antimicrobial effect of *Sophora angustifolia* extracts against food-borne pathogens. First, *Sophora angustifolia* was extracted with methanol at room temperature, and the methanol extracts from *Sophora angustifolia* were fractionated using petroleum ether, chloroform, ethyl acetate, and methanol. The antimicrobial activity of the *Sophora angustifolia* extracts was determined using the paper disc method against food-borne pathogens and food spoilage bacteria. The methanol extracts of *Sophora angustifolia* showed the highest antimicrobial activity against *Staphylococcus aureus* and *Salmonella typhimurium*. A synergistic effect was found in the combined extracts of *Sophora angustifolia* and *Portulaca oleracea*, compared to the activity of each extract alone. Finally, the growth inhibition curve was determined using the methanol extracts of *Sophora angustifolia* against *Staphylococcus aureus* and *Salmonella typhimurium*. The methanol extract of *Sophora angustifolia* showed strong antimicrobial activity against *Staphylococcus aureus* at a concentration of 5,000 ppm. The 5,000 ppm methanol extract from *Sophora angustifolia* retarded the growth of *S. aureus* for more than 24 hours and of *Salmonella typhimurium* for up to 12 hours.

Keywords: *Sophora angustifolia*, antimicrobial activity, food-borne pathogens

Introduction

The deterioration or spoilage of food is mainly caused by the growth of microorganisms. To maintain product consistency, an array of preservation and storage techniques are used, of which heat treatment, freezing and refrigeration are the most commonly used. Heat treatment is one of the major forms of food preservation despite its limited appeal in terms of freshness and taste. When food is cooked, changes in texture and flavor reduce freshness and impair taste. Freezing is a more effective method to retain freshness and taste but not the best to store food over the long term. To make up for the shortfalls of these food preservation methods, the food industry uses preservatives to prevent or inhibit microbial growth. Most food preservatives are chemically synthesized and therefore cause concern with respect to their side effects, although their effectiveness is very high. Health issues such as chronic toxic effects, carcinogenic potential and mutation induction have emerged concerning the accumulation of chemical preservatives in the body (1). As consumers become more health-conscious and alert to chemical preservatives, food manufacturers are moving toward seeking natural substitutes for chemical preservatives.

The applications of natural antimicrobial properties identified in the areas of Chinese herbal medicine and folk medical practices for food preservation have been studied for a long time, and the research interest continues to grow (2-7). Among the plants that contain antimicrobial substances is *Sophora angustifolia*, which grows naturally in many countries, including Korea and China. *Sophora angustifolia* has matrine, sophocarpine and isoprenyl chalcone as its major substances and has a bitter taste. With biocidal properties, the plant is widely used as an ingredient of traditional medicine (8). In Korea, the plant is one of the key ingredients in Chinese herbal medicine and folk medicine administered for the relief of pain, antitoxic effects, anticancer and gynecological illness. This study aimed to assess the antimicrobial properties of *Sophora angustifolia* against food contaminant bacteria. Dried *Sophora angustifolia* was systematically extracted and fractionated using various organic solvents. The resultant antimicrobial compounds were tested against food contaminant bacteria. In addition, the antimicrobial effects of *Sophora angustifolia* combined with *Portulaca oleracea*, another indigenous plant used for Chinese herbal medicine, were investigated.

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Materials and Methods

Experimental materials The experimental material, dried *Sophora angustifolia,* is an indigenous plant, purchased from a herbal medicine street in Daegu city, located in the southern part of South Korea, in December 2003. After impurities were removed from the plant, it was washed lightly with water, dried and then ground into powder for extraction.

Strains and culture A total of nine bacteria strains (two Gram positive and seven Gram negative) were from the Bioengineering Research Center of the Korean Institute of Science and Technology Information (Table 1). All strains were cultured on Tryptic Soy Broth (TSB, Difco, Caldo, USA) at 37°C for 18 to 24 hours. The solid culture medium used for the antimicrobial experiment was Tryptic Soy Agar (TSA, Difco, Caldo , USA).

Extraction of antimicrobial substances Dried *Sophora angustifolia* (500 g) was extracted twice with the same

Table 1. List of microorganisms used for antimicrobial activity test

	Strains
Gram positive bacteria	*Staphylococcus aureus* ATCC 25923
	Staphylococcus epidermidis ATCC 26734
Gram negative bacteria	*Escherichia coli* ATCC 25922
	Pseudomonas aeruginosa ATCC 27853
	Salmonella typhimurium ATCC 14028
	Salmonella enteritidis ATCC 13076
	Shigella sonnei ATCC 25931
	Shigella dysenteriae ATCC 9199
	Shigella flexneri ATCC 12022

volume of petroleum ether, chloroform, ethyl acetate and methanol. The powdered *Sophora angustifolia* was poured into a tube to which 1L of methanol was added and then kept at room temperature for 6 hours. The mixture was filtered through Whatman No.2 filter paper (Whatman International Ltd., England) to remove impurities. The filtrate was concentrated in a vacuum concentrator (EYELA, N-N. Series, Japan) at 45°C. The concentrated methanol extract was systematically fractionated using different solvents - petroleum ether, chloroform, ethyl acetate and methanol. The fractionation procedure involved pouring methanol extract and these solvents into a separatory funnel and shaking the mixture manually for five minutes. The mixture was left at room temperature for 15 minutes before fractionation. The water extract of *Sophora angustifolia* was obtained with organic solvents. Distilled water was added to the residue, which was then boiled at 100°C for 30 minutes and filtered in the same way as the methanol extract. The culture filtrate was concentrated in a vacuum concentrator (EYELA, N-N. Series. Japan) at 45°C and diluted with 70% ethanol to be used in the experiment.

Antimicrobial activity of *Sophora angustifolia* The antimicrobial activity of extracts of *Sophora angustifolia* was evaluated using paper disc (9). All experiments were duplicated twice. The evaluation procedures were performed by placing an equal amount of bacterial strains cultured in TSB onto a TSA plate according to the pour-plate method. The optical density of the culture (0.4) was measured using a spectrophotometer (Nontron instruments, Italy) at 620 nm. The plate was hardened at room temperature. A sterile paper disc was placed onto the surface of the plate so that 20 μl of each sample of petroleum ether extract, chloroform extract, ethyl acetate extract, methanol extract and water extract would diffuse into the water at concentrations ranging from 100 ppm to 1,000 ppm. The procedure was repeated for the *Sophora angustifolia*-free control sample containing 70% ethanol. After 24-hour incubation at 37°C, the radius of clear zone (mm) surrounding each disc was measured to evaluate the inhibitory activity.

Enhanced antimicrobial effect when combined with *Portulaca oleracea* The *Sophora angustifolia* extract was combined with another antimicrobial plant extract, *Portulaca oleracea*, to evaluate the antimicrobial activity of the combined substance. The 250 ppm methanol extracts of *Portulaca oleracea,* whose antimicrobial properties were proven in preliminary tests, was well mixed with 250 ppm *Sophora angustifolia*. The inhibitory activities of ethyl acetate extracts of *Sophora angustifolia* (500 ppm) and *Portulaca oleracea* (500 ppm) were compared.

Growth curve of test microorganisms The methanol extract of *Sophora angustifolia* was sterilized through filtration using a 0.2 μm membrane filter (Toyo Roshi Kaisha. Ltd., Japan). Each extract was added to liquid culture medium at 3,000, 4,000 and 5,000 ppm. Samples was sterilely inoculated with bacterial culture fluid diluted 1:1,000,000 to an optical density of 0.4 and then cultured at 37°C for 72 hours. The optical density of the culture was measured at 620 nm every 12 hours using a spectrophotometer. Gram positive *Staphylococcus aureus* and gram negative *Salmonella typhimurium* were used to determine whether isolates of *Sophora angustifolia* inhibited the growth of these two different bacteria species. The inhibitory effects were determined by comparing the optical density at different concentration levels (10).

Results and Discussion

Extraction yield *Sophora angustifolia* extract was partitioned using petroleum ether, chloroform and ethyl acetate, and the obtained yields are presented in Table 2. The extraction yields for *Sophora angustifolia* fractions based on petroleum ether, chloroform, ethyl acetate, methanol and water were 5.7%, 8.9%, 7.6%, 13.5% and 23.7%, respectively. Petroleum ether extract showed the lowest yield and water extract the highest.

Antimicrobial activity of *Sophora angustifolia* The antimicrobial activity of *Sophora angustifolia* was evaluated by applying its various organic and aqueous fractions to food spoilage and pathogenic bacteria species using paper discs. As a result, the inhibitory activity of *Sophora angustifolia* extracts produced by the four solvent-based extractions and by the water-based extraction increased with increasing concentration of *Sophora angustifolia* extracts that diffused through the discs, as shown in Table 3. Thus there was a significant correlation between concentration level and inhibitory activity measured by the size of clear zone. The methanol extract displayed the largest clear zone (22 mm) against *Staphylococcus aureus* at 1,000 ppm concentration (Fig. 1). The inhibitory responses of *Sophora angustifolia* extracts to indicator

Table 2. Yield of antibacterial substances from *Sophora angustifolia* by extraction solvents

Fraction	Dry weight (g)	Yield (%)
Petroleum ether extract	28.5	5.7
Chloroform extract	44.5	8.9
Ethyl acetate extract	38.1	7.6
Methanol extract	67.5	13.5
Aqueous extract	118.5	23.7

Table 3. Antimicrobial activities of each solvent fraction from *Sophora angustifolia* against Gram positive bacteria

Strians	Clear Zone (mm)[a]					
	Fraction conc.(ppm)	PE	C	EA	M	A
Staphylococcus aureus	100	-[b]	-	-	-	-
	250	8	9	12	14	-
	500	9	10	13	18	-
	1,000	10	11	15	22	-
Staphylococcus epidermidis	100	-	-	-	-	-
	250	6	9	15	13	-
	500	8	10	16	15	-
	1,000	10	12	18	16	-

a) Diameter, b) No inhibitory zone was formed
PE : Petroleum ether extract C : Chloroform extract
EA : Ethyl acetate extract M : Methanol extract
W : Water extract

bacterium varied depending on the culture medium and concentration. The petroleum ether extract of *Sophora angustifolia* mainly inhibited the growth of *Shigella sonnei*, while the chloroform extract of *Sophora angustifolia* most effectively reacted against *Shigella dysenteriae*. Meanwhile, the methanol extract of *Sophora angustifolia* was active in inhibiting the growth of all the bacteria tested in the study, and demonstrated an inhibitory activity even at 250 ppm concentration.

Many broadleaved trees are used as ingredients of Chinese herbal medicine. These broadleaved trees have a more complex and broader range of organic compounds, compared to coniferous trees; thereby contributing to their therapeutic use. Bae (11) reported the efficacy of flavan and procyanidin extracted from the bark of Blackjack oak. *Sophora angustifolia* has an intensely bitter taste and its derived substances such as sophocarpine and isoprenyl chalcone are known for inhibiting the growth of micro-organisms (8). Amid increasing interest in the therapeutic properties of plants and trees, many investigators have conducted research on the post-ingestive effects of plant extracts. Row (12) defined coumarin as lactone created from o-hydroxy cinnamic acid on the ring closure reaction between phenol and the carboxyl group, and cited various types of coumarin derivatives. Harbone (13) examined the proton peak of glucose.and Markham (14) identified glucose present in Kaempferol. Sen (15) examined the efficacy of flavonol glycoside after isolating it from *Calotropis gigantea.*

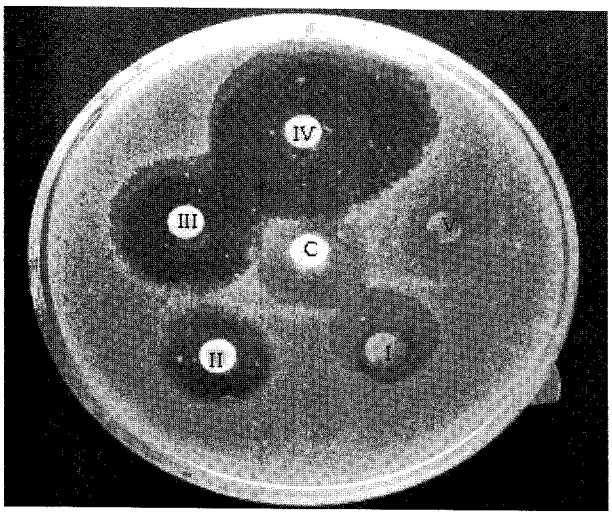

Fig. 1. Antimicrobial activities of various extracts of *Sophora angustifolia* against *Staphylococcus aureus* at the concentration of 1,000ppm.
C: control (70% ethanol), I: petroleum ether extract
II: chloroform extract, III: ethyl acetate extract
IV: methanol extract, V: Aqueous extract

In the present study, isolates of *Sophora angustifolia* showed significant antimicrobial activity against all of the gram negative bacteria, as presented in Table 4. The methanol extract of *Sophora angustifolia* showed a 22 mm clear zone of inhibition by reacting most effectively against *Salmonella typhimurium* at 1,000 ppm concentration. At the same time, the ethyl acetate extract of *Sophora angustifolia* showed a wider spectrum of antimicrobial activity by restricting the growth of both gram positive and gram negative bacteria species. Kim et al. (16) stated that the methanol extract of *Zanthoxylum schinifolium* was more effective to quell gram negative bacteria *E. coli* than gram positive bacteria. Meanwhile, the evaluation of antimicrobial activity of *Sophora angustifolia* extract could not be performed at concentrations of 100 ppm or lower. Water extract of *Sophora angustifolia* did not show significant antimicrobial activity against any of the bacteria tested. Methanol extracts obtained from medicinal plants and trees are known to have saponin, organic compounds, tannin, glucose, glycoside and other alkaloid components. A majority of these properties were also identified in the most antimicrobial methanol extract of *Sophora angustifolia.*

Enhanced antimicrobial effect when combined with *Portulaca oleracea* The antimicrobial effect of the methanol extract of *Sophora angustifolia* significantly increased when combined with the methanol extract of *Portulaca oleracea*, and this enhancement was especially strong against *Staphylococcus aureus,* as presented in Table 5. The methanol extract of *Sophora angustifolia* alone exhibited a 16 mm zone of inhibition against the abovementioned bacteria at 500 ppm concentration, whereas the combined methanol extract showed a 22 mm clear zone of inhibition at the same concentration level (250 ppm each). The combined methanol extract also

Table 4. Antimicrobial activities of each solvent fraction from *Sophora angustifolia* against Gram negative bacteria.

Strains	Clear zone on plate(mm)[a]				
Strains	Fraction conc.(ppm)	PE	C	EA	M	W
Escherichia coli	100	-[b]	-	-	-	-
	250	8	9	10	11	-
	500	9	10	11	13	-
	1,000	10	11	12	15	3
Pseudomonas aeruginosa	100	-	-	-	-	-
	250	3	-	10	12	-
	500	4	6	11	15	-
	1000	5	8	12	17	2
Salmonella typhimurium	100	-	-	-	-	-
	250	8	9	13	16	-
	500	11	10	15	18	-
	1,000	12	11	16	20	3
Salmonella enteritidis	100	-	-	-	-	-
	250	7	7	9	13	-
	500	8	9	12	17	-
	1,000	10	11	15	19	4
Shigella sonnei	100	-	-	-	-	-
	250	9	8	9	12	-
	500	10	9	10	14	3
	1,000	13	10	11	18	4
Shigella dysenteriae	100	-	-	-	-	-
	250	7	9	10	13	-
	500	8	11	13	15	-
	1,000	9	12	15	18	3
Shigella flexneri	100	-	-	-	-	-
	250	9	8	12	14	-
	500	11	9	14	17	-
	1,000	12	10	15	19	5

a) Diameter b) No inhibitory zone was formed
PE : Petroleum ether extract C : Chloroform extract
EA : Ethyl acetate extract ME : Methanol extract W : Water extract

Table 5. Antimicrobial activity of combined extracts from *Sophora angustifolia* and *Portulaca oleracea*

Strains	Clear zone on plate(mm)[1]			
Strains	control	*Sophora angustifolia* (500 ppm)	*Portulaca oleracea* (500 ppm)	Both[3] (each 250 ppm)
Staphylococcus aureus	-[2]	16	19	22
Shigella dysenteriae	-	15	17	19

[1]Diameter
[2]No inhibitory zone was formed
[3]*Sophora angustifolia* and *Portulaca oleracea*

demonstrated a greater antimicrobial activity against *Shigella dysenteriae* than the methanol extract of *Sophora angustifolia* (Fig. 2). Since the safety and medicinal properties of extracts obtained from plants and trees are not officially recognized, there has not been active research on the medicinal effects of tree extracts in western countries. Nevertheless, research efforts to prove the antimicrobial properties of traditional medicinal plants and trees would speed up the development of effective natural food preservatives.

Impact of methanol extract of *Sophora angustifolia* on gram-negative and gram-positive bacterial species The methanol extract of *Sophora angustifolia* was added into TSB at different concentrations of 0 ppm, 3,000 ppm, 4,000 ppm and 5,000 ppm, inoculated with gram positive *Staphylococcus aureus* and gram negative *Salmonella typhimurium* and then cultured for 72 hours. The measurement of bacterial growth over this period produced the growth curve presented in Figs. 3 and 4.

The population of *Staphylococcus aureus* rapidly increased in the control tube that didn't contain the ethyl acetate extract of *Sophora angustifolia* in as little as nine hours after culture. However, the bacterial growth was suppressed for 24 hours, and the spread of the bacteria was slowed with the 5,000 ppm methanol extract of *Sophora angustifolia* (Fig. 3) Chung (17) stated that the ethanol extract of palm cactus slowed down the growth of *Staphylococcus aureus* at a concentration of 3.0 mg/ml or higher. Jeon et al. (18) also reported that the methanol extract of plantain quelled the growth of *Staphylococcus aureus*. The present study also demonstrated that the methanol extract of *Sophora angustifolia* inhibited the growth of the same bacteria. The inhibitory activity of the

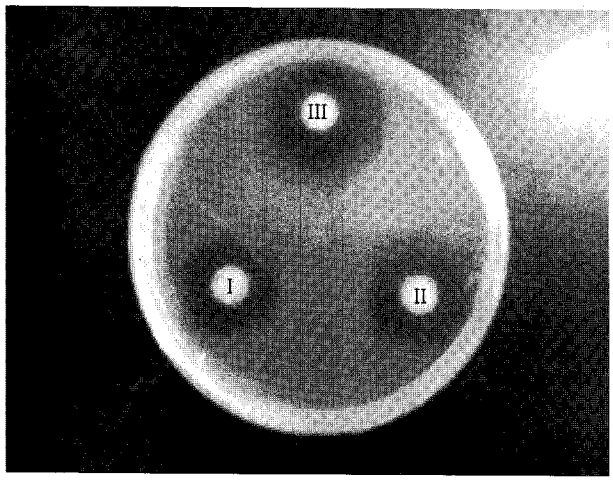

Fig. 2. Antimicrobial activities of methanol extract of *Sophora angustifolia*, methanol extract of *Portulaca oleracea* and both extracts combined against *Shigella dysenteriae*
I: *Sophora angustifolia* (500 ppm)
II: *Portulaca oleracea* (500 ppm)
III: *Sophora angustifolia* (250 ppm) and *Portulaca oleracea* (250 ppm)

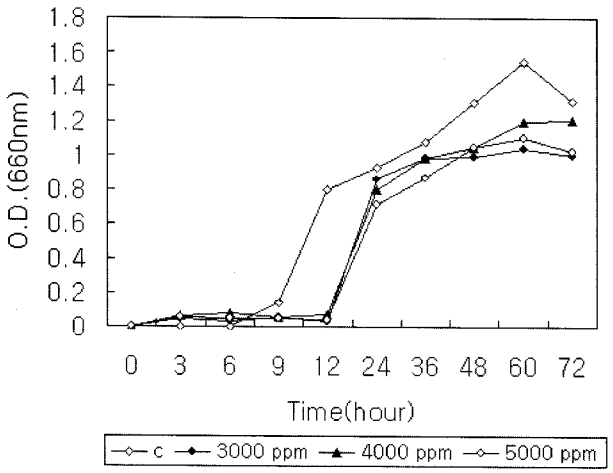

Fig. 3. Effect of methanol extracts of *Sophora angustifolia* against the growth of *Staphylococcus aureus*. (◆), control ; (■), 1000 ppm; (▲), 3000 ppm; (●), 5000 ppm

methanol extract against *Salmonella typhimurium* was observed in the same way over the period of 72 hours, and the results are shown in Fig. 4. A rapid growth of bacteria was observed in the control tube at six hours after culture, whereas the 5,000 ppm methanol extract of *Sophora angustifolia* effectively restricted the proliferation of *S. typhimurium* for 12 hours following culture. Shin et al. (19) claimed that the ethanol extract of *Perilla frutescens* inhibited the growth of *S. typhimurium* for 36 hours. Chung et al. (20) asserted that mushroom extract showed high inhibitory activity against the same bacteria. It is therefore evident that there are many plants and trees that have been proven to have antimicrobial activity against food-borne pathogenic bacteria, supporting the possible applications of their extracts as food preservatives.

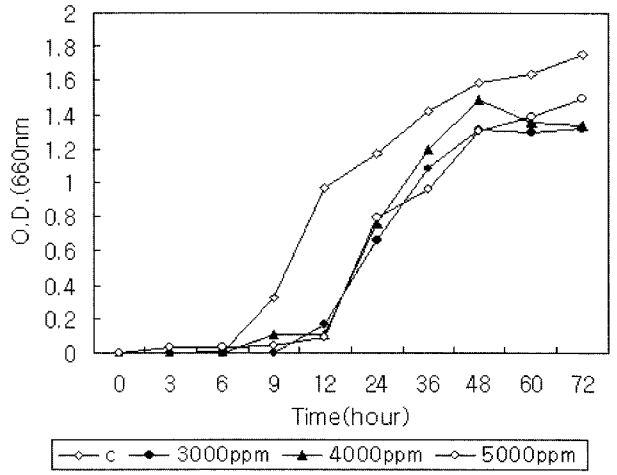

Fig. 4. Effect of methanol extracts of *Sophora angustifolia* against the growth of *Salmonella* Typhimurium. (◆), control ; (■), 1000 ppm; (▲), 3000 ppm; (●), 5000 ppm

Conclusion

This study aimed to investigate the antimicrobial activity of *Sophora angustifolia*, a native plant widely used in Korean folk medicine and Chinese herbal medicine, against food-borne pathogens in an effort to promote the development of natural resource-based, food preservatives. *Sophora angustifolia* was systematically fractionated using petroleum ether, chloroform, ethyl acetate and methanol at room temperature. The water extract was also obtained. The inhibitory activity of each solvent- and water-based extract was evaluated against a total of nine food-borne, pathogenic bacteria: *Staphylococcus aureus, Staphylococcus epidermidis, Salmonella enteritidis, Shigella flexneri, Escherichia coli, Salmonella typhimurium, Shigella dysenteriae, Pseudomonas aeruginosa and Shigella sonnei.* The methanol extract showed the largest clear zone of inhibition in the examination of effects of different concentrations. Among all the bacteria tested, *Staphylococcus aureus* and *Salmonella typhimurium* were the most sensitively reacted. When combined with the methanol extract of *Portulaca oleracea*, the combined extract demonstrated a greater inhibitory activity than the methanol extract of *Sophora angustifolia* alone. At the same time, it was found that the methanol extract of *Sophora angustifolia* suppressed the growth of *Staphylococcus aureus* and *Salmonella typhimurium* for 24 hours and 12 hours, respectively, after culture at 5,000 ppm concentration.

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