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Inhibitory Effect of Mugwort(*Artemisia asiatica* Nakai) on the Growth of Food Spoilage Microorganisms and Identification of Antimicrobial Compounds

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Abstract

The antimicrobial activity of mugwort(*Artemisia asiatica* Nakai) was investigated. The methanol extract of dried mugwort was fractionated to hexane, chloroform, ethylacetate, butanol, and aqueous fractions. The hexane fraction among these fractions showed the highest inhibitory effect on the growth of microorganisms such as *Bacillus subtilis, Escherichia coli, Staphylococcus aureus* and *Lactobacillus plantarum. Bacillus subtilis, Escherichia coli,* and *Staphylococcus aureus* were completely inhibited at a concentration of 250, 500, and 750μg/ml respectively. The hexane fraction was further fractionated into 16 subfractions by silica gel column and thin layer chromatography(TLC). The subfraction No. 8, 9, and 10 on TLC exhibited high antimicrbial activity. At 3rd fractionation, subfraction No. 2 inhibited the growth of microorganisms at 500μg/ml. Heptadecane, dodecamethyl cyclohexasiloxane, (E,E)-2,4-decadienal, dodecamethyl pentasiloxane, coumarin, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone, neophytadiene, tridecanoic acid, methyl ester, 2-methyl-4,5-nonadiene, (Z,Z)-9,12-octadecadienoyl chloride, and bis(2-ethylhexyl) phthalate were identified from this antimicrobial fraction by GC-MS.

Key words: antimicrobial activity, mugwort, coumarin

INTRODUCTION

Herbs and spices have been used to extend the shelf life of foods and their antimicrobial compound have received much attention in recent times(1-9).

Mugwort(*Artemisia asiatica* Nakai) taxonomically belong in the Carduaceae family as a perennial plant and is widely distributed in Japan, China and Europe as well as Korea(10). In oriental countries including Korea, mugwort has been used as a therapeutic agent in the treatment of nosebleeding and uterine hemorrhage, an anodyne, phthisis, asthisis, chronic bronchitis and chronic digestive ailments(11). Also, it has long been used as a food additive of various food stuffs(12). Flavour compound or essential oil of mugwort, as other spices, is known to have biological activity of an antimicrobial and antitumor, etc.(13). However, the active compounds or mechanism as a food preservative are not clearly evaluated. In this study, this spice is screened with high antimicrobial effect, and chemical studies carried out to search for substance with inhibitory effect for food spoilage microorganisms. After extraction, fractionation and isolation of antimicrobial compounds by column chromatography and TLC, identification of compounds in antimicrobial fraction by GC-MS was done.

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MATERIALS AND METHODS

Extraction and fractionation

Raw mugwort leaves were purchased at Susan, Kyung Sang Nam-Do in June 1995. Dried and then powdered leaves(4kg) were extracted with MeOH at 80°C under reflux for 3hrs. After removal of the precipitate by filteration, it were concentrated with a vacuum rotary evaporator(Eyela N-N) at 70°C. The concentrated extract (967.41g) was further fractionated into hexane(107.67g), chloroform(26g), ethylacetate(43.2g), butanol(131.19g) and aqueous(398.20g) successively(Fig. 1).

Screening for antimicrobial activity

Bacillus subtilis ATCC 6633, *Staphylococcus aureus* ATCC 13301, *Lactobacillus plantarum* KCTC 3108 and *Escherichia coli* ATCC 1129 were used as test organisms.

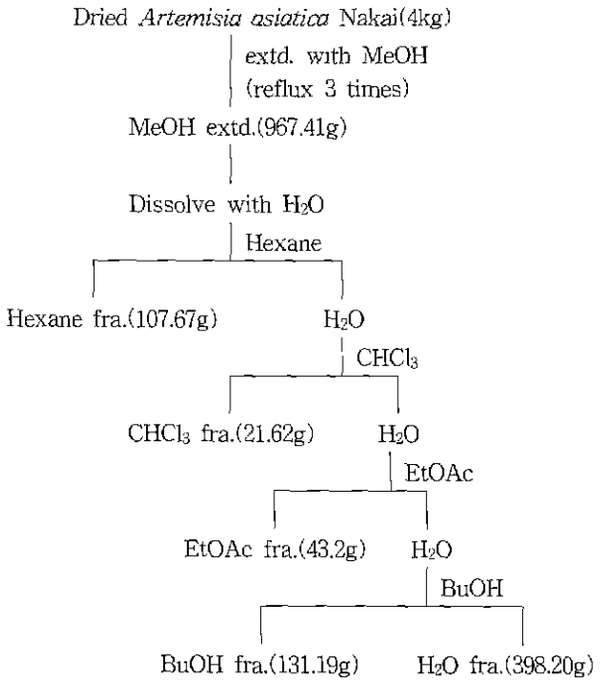

Fig. 1. Solvent fractions from methanol extract of mugwort(*Artemisia asiatica* Nakai)

Lactobacillus MRS broth and agar for *L. plantarum* and tryptic soy broth and nutrient agar for other orga nisms were used. The broth medium was dispensed 300ml quantities in 500ml flask and sterilized by autoclaving at 121°C for 15min. Test tubes each containing 10ml of liquid culture were inoculated 1ml of suspension of the test organisms. The suspension was uniformly spread over the medium using a glass rod. The inoculated test tubes were then incubated at 37°C for 24hrs. The suspension(10^5CFU/ml) was inoculated 0.1ml in each fractionated extract at a various concentration of 250, 500, 750 and 1,000μg/ml. After incubated at 37°C for 24hrs optical density(O.D.) was checked at 660nm with a spectrophotometer.

Silica gel chromatography

The hexane fraction was further fractionated using silica gel chromatography(Fig. 2). The sample(96g) was mixed with silica gel and placed on the column I(10cm×1m) packed with silica gel(1.5kg). The sample was eluted with hexane and ethylacetate and each 50ml of eluent was collected in each flask. After testing antimicrobial activity of eluent in each flask, eluents that had high inhibitory effects were combined, and then refractionated by silica gel column II(2.5cm×60cm).

Thin layer chromatography

The fractions collected from silica gel column chromatography(column II) showing antimicrobial activity were further fractionated on TLC plate(Art. 5721, DC-Fertigplatten Kieselgel 60). The plates were developed with 80% hexane solution(Hexane : EtOAc= 8 : 2 v/v).

Separation and identification of compounds in antimicrobial fraction by GC-MS

GC-MS analysis of antimicrobial fraction was carried out with a HP-5988 mass spectrometer connected with

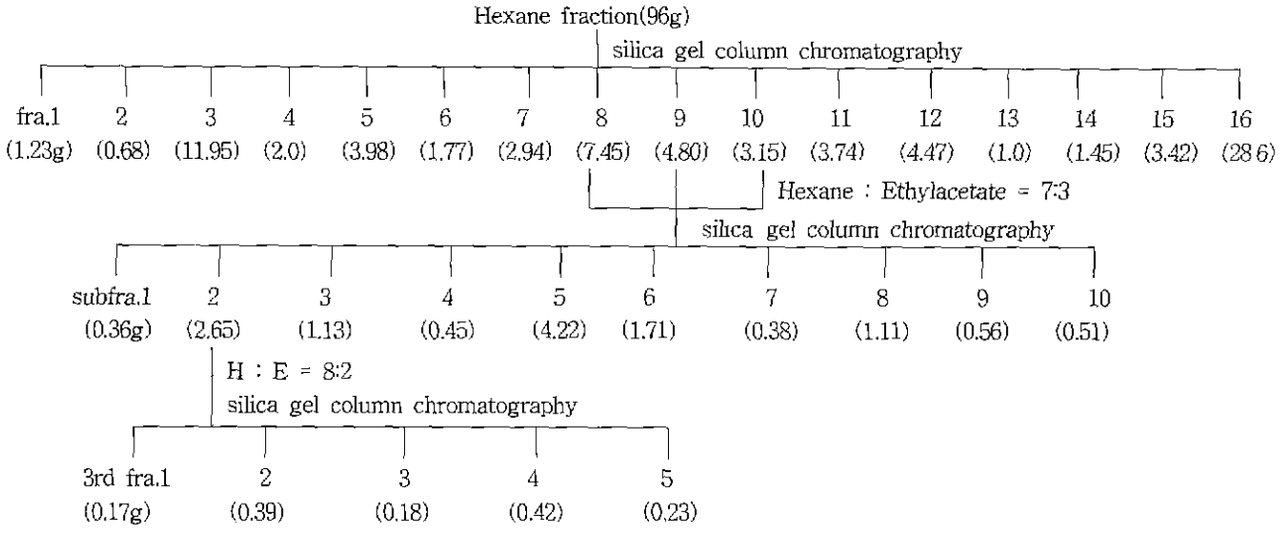

Fig. 2. Fractionation and isolation of hexane extract of mugwort(*Artemisia asiatica* Nakai).

HP-5890 II Gas chromatography using a bonded polyethylene glycol fused silica capillary column(50m×0.2mm×0.11μm). The mass spectra were recorded at a electron energy of 70eV and the ion source temperature was 150°C. The column was operated with a temperature program from 60°C to 300°C at 10°C/min, and then held for 4 min at 300°C. Helium was used as a carrier gas(0.5ml/min, splitless).

RESULTS AND DISCUSSION

Antimicrobial activity of extract fractions

The methanol extract was tested on four species of food spoilage microorganisms *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Lactobacillus plantarum* at a various concentration of 250, 500, 750 and 1000μg/ml. The methanol extract showed complete inhibition on the growth of *B. subtilis* and *S. aureus* at a concentration of 250μg/ml and 86.56% for *E. coli*(Table 1). It showed stronger inhibitory effect than that of sodium propionate which has been used as a antimicrobial agent. Sodium propionate showed complete inhibition on the growth of *B. subtilis, E. coli* and *S. aureus* at 5% concentration. And *L. plantarum* was inhibited 50.87% at the same concentration(Table 2). Methanol extract was further fractionated into hexane, chloroform, ethylacetate, butanol and aqueous. The hexane fraction among these fractions showed the highest inhibition effect against all four test strains. *B. subtilis, E. coli*, and *S. aureus* were completely inhibited at 250, 500, and 750μg/ml respectively and *L. plantarum* was inhibited 56.43% at 1000μg/ml. Chloroform fraction completely inhibited the growth of *B. subtilis* at 750μg/ml, *E. coli* and *S. aureus* at 1000μg/ml. Ethylacetate, butanol and aqueous fraction did not show antimicrobial activity on three test microorganisms except *L. plantarum*. This result is in agreement with that of Kim et al.(13) who reported the effect of hot water extract from mugwort on the growth of *E. coli*. We suggest that the major compound which showed antimicrobial activity of mugwort is a nonpolar

Table 1. Antimicrobial activity of methanol extract and solvent fractions from methanol extract of mugwort on the growth of *B. subtilis, E. coli, L. plantarum* and *S. aureus*

Fractions	Concentration (μg/ml)	Antimicrobial effect(%)			
		B. subtilis	*E. coli*	*L. plantarum*	*S. aureus*
Methanol	1000	100	100	50.59	100
	750	100	100	51.22	100
	500	100	99.16	50.34	99.56
	250	100	86.65	45.65	99.20
Hexane	1000	100	100	56.43	100
	750	100	100	49.50	100
	500	100	100	47.01	97.62
	250	100	76.09	43.99	92.46
Chloroform	1000	100	100	76.93	100
	750	100	100	67.77	98.41
	500	99.79	100	58.19	98.21
	250	48.88	98.20	55.49	100
Ethylacetate	1000	0	0	63.78	0
	750	0	0	61.18	0
	500	0	0	58.95	0
	250	0	0	64.95	0
Buthanol	1000	0	0	75.45	0
	750	0	0	68.56	0
	500	0	0	66.93	0
	250	0	0	62.85	0
Aqueous	1000	0	0	85.80	13.49
	750	0	0	78.50	7.14
	500	0	0	65.88	6.11
	250	0	0	65.17	3.57

Table 2. Antimicrobial activity of sodium propionate on the growth of *B. subtilis*, *E. coli*, *L. plantarum* and *S. aureus*

Concentration (%)	Antimicrobial effect(%)			
	B. subtilis	*E. coli*	*L. plantarum*	*S. aureus*
0.5	8.82	23.68	0.97	16.90
1.0	33.97	47.74	4.58	42.96
3.0	68.83	71.19	8.89	91.32
5.0	100	100	50.87	100

Table 3. Antimicrobial activity of 3rd-fractions from hexane extract of mugwort on the growth of *B. subtilis*, *E. coli*, *L. plantarum* and *S. aureus*

Fraction No.	Concentration (μg/ml)	Antimicrobial effect(%)			
		B. subtilis	*E. coli*	*L. plantarum*	*S. aureus*
1	500	100	100	72.82	56.16
	250	99.77	100	67.34	49.08
2	500	100	100	98.22	100
	250	100	99.34	85.16	98.97
3	500	100	100	79.00	100
	250	100	100	86.87	88.81
4	500	100	100	87.77	100
	250	96.26	100	59.34	100
5	500	100	100	89.19	100
	250	97.17	42.21	71.17	96.83

Table 4. Compounds identified from antimicrobial fraction No. 2 of silica gel column chromatography of hexane extract of mugwort by GC-MS

Retention time(min.)	Compounds	Molecular weight	Formular	Area (%)
15.627	Heptadecane	240	$C_{17}H_{36}$	2.65
18.697	Dodecamethyl cyclohexasiloxane	444	$C_{12}H_{36}O_6Si_6$	6.26
19.091	(E,E)-2,4-decadienal	152	$C_{10}H_{16}O$	2.36
21.366	Dodecamethyl pentasiloxane	384	$C_{12}H_{36}O_4Si_5$	3.67
21.501	Coumarin	146	$C_9H_6O_2$	23.3
22.836	5,6,7,7a-tetrahydro-4,4,7a-trimethyl 2(4H)-benzofuranone	180	$C_{11}H_{16}O_2$	2.34
26.375	Neophytadiene	278	$C_{20}H_{38}$	2.83
26.804	Tridecanoic acid, methyl ester	228	$C_{14}H_{28}O_2$	4.32
28.936	2-Methyl-4,5-nonadiene	138	$C_{10}H_{18}$	7.86
29.859	(Z,Z)-9,12-octadecadienoyl chloride	298	$C_{18}H_{31}ClO$	3.35
36.961	Bis(2-ethylhexyl) phthalate	390	$C_{24}H_{38}O_4$	4.03

compound.

Antimicrobial activity of hexane fraction

Further separation of the hexane fraction was carried out using silica gel column chromatography and thin layer chromatography(Fig. 2). In the first fractionation, fraction No. 8, 9 and 10 showed the strong antimicrobial activity at 500μg/ml. These fractions(8~10) collected from the first fractionation, were combined, concentrated and then refractionated. Fraction number 2 from the second fractination showed the most strong antimicrobial activity at 500μg/ml(data not shown). No. 2(antimicrobial compound) was separated from five fraction using TLC. Table 3 is the result of the inhibitory effect of these five fraction. All five fractions completely inhibited the growth of *B. subtilis* and *E. coli* at a level of 500μg/ml. No. 2 fraction among these fractions had inhibitory effects on the growth of

B. subtilis, E. coli, and *S. aureus*. Growth was completely inhibited at a level of 250μg/ml and *L. plantarum* was inhibited 98.22% at 500μg/ml.

Fraction No. 2 was identified by GC-MS(Table 4). Among these identified 11 compounds, coumarin was the major constituent of the antimicrobial compounds (23.39%), heptadecane, dodecamethy cyclohexasiloxane, (E,E)-2,4-decadienal, dodecamethyl pentasiloxane, 5,6, 7,7a-tetrahydro-4,4,7a-trimethyl 2(4H)-benzofura-none, neophytadiene, tridecanoic acid, methyl ester, 2-methyl -4,5-nonadiene, (Z,Z)-9,12-octadecadienoyl chloride, and bis(2-ethylhexyl)phthalate were found in small quantities(2.34~7.86%). Frank et al.(2) reported that the inhibitory effects of the oils were mainly due to the most abundant components. Kim et al.(13) reported that among the major volatile compounds(myrcene, cineole, 2-pyrrolidinone, camphor, thujone, 1-acetyl-piperidine, caryophyllene, coumarin, and farnesol) identified from raw mugwort or mugwort tea, 1% of coumarin showed high antimicrobial effect against *E. coli*. Also, Vichkonova et al.(14) reported that natural coumarin have antimicrobial activity and antiviral activity. Therefore, we suppose that the major antimicrobial compound of the mugwort's essential oils is coumarin.

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