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Antimicrobial activities in the Korean Traditional Leaf Mustard, *Brassica juncea* Coss.

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ABSTRACT

As part of developing natural food preservatives, the antimicrobial effect of ethanol and water extracts from the *Cruciferous* vegetable was examined Korean traditional *Brassica juncea* Coss. was used widely as an ingredient of *Kimchi*, a natural flavoring and spice for a long time. Antimicrobial activities were examined against 15 microorganisms which were food-born pathogens and/or food poisioning microorganisms and food-related bacteria and yeasts. Ethanol extract from leaf mustard showed an antimicrobial effect in most of the strains used in the present study. The lowest minimum inhibitory concentration (MIC) were seen in *Bacillus subtilis* and *Bacillus natto* at 10 mg/ml. MIC of water extract was 40-60 mg/ml for bacteria and yeast. Antimicrobial activity of the ethanol extract was not disappeared by the heating at 121℃ for 15 min and not affected by pH.

Key words : Antimicrobial activity, minimum inhibitory concentration (MIC)

INTRODUCTION

Leat mustard is a *cruciferous* vegetable whose seed in powder form has been used as mustard spice in the US, Europ and Japan (Farrell, 1985). Originated from China, it is cultivated widely in Korea and Japan. The Korean variety, *Brassica juncea* Coss., is used not only as a spice but also as an ingredient of nappa cabbage *Kimchi*, or as the main ingredient of *Kimchi*, due to its unique taste and flavor (Kim and Kim, 1987). When used as a *Kimchi* ingredient, it is a good source of minerals including abundant Ca and K and is known to prolong the storage period of *Kimchi* due to its slow fermentation speed, and to maintain stable color during a long storage period. Leaf mustard contains the allylisothiocyanate (AIT) glucosinolate called sinigrin, which gives a unique hot flavor. Sulfur compounds and related compounds are produced by this glycoside due to myrosinase action (Morimoto *et al.*, 1983). Sulfur compounds which have a high reactivity change themselves due to other organic sulfur compounds and are reported to contain many physiologically active substances having antimicrobial, anti-fungal, and anti-coagulative effects (Eric, 1985; Bordia *et al.*, 1997).

Studies on leaf mustard include studies on antioxidative substances in leaf mustard and mustard (Han *et al.* 1987), the purple color antocyanin in leaf mustard (Park, 1979a; Park, 1979b), nutrient contents in

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leaf mustard (Morimoto *et al.*, 1983; Saito and Iwasaki, 1980), volatile compounds in leaf mustard (Kameoka and Hashimoto, 1980), the types and content of glucosinolate (Curitis *et al.*, 1987; Diana *et al.*, 1987; Shen, 1987), genetic analysis (Reddy *et al.*, 1988) and enzymes (Kumar and Gupta, 1987).

Most of these studies on leaf mustard were on nutrition or food compounds, and studies are rare on antimicrobial effects in leaf mustard. Therefore, as part of developing natural food preservatives, ethanol and water were extracted from the Korean leaf mustard *Brassica juncea* Coss. which is believed to have antimicrobial effects. This extracts were tested for antimicrobial effects against 15 different microorganisms including pathogens, food-born microorganisms, and food related bacteria and yeasts, heat and pH stability of the antimicrobial substances in the extracts.

MATERIALS AND METHODS

Plant materials

The Korean traditional leaf mustard, *Brassica juncea* Coss. was collected in Suncheon, in the south of Korea in 2002. The plants were washed in tap water, and milled after being dried in the dark.

Microbial strains and reagents used in experiments

Tabel 1 shows the list of strains used. The nutrient media was purchased from Difco Co. (U.S.A.).

The preparation of extracts

Water extract

The aqueous extracts were made as follow : 1Kg of leaf mustard were added with three times of distilled water, homogenized with homogenizer for 5 minutes and shaked at room temperature for 24 hrs. 3 ℓ of distilled water was added to 1st extract and treated same method as 1st extraction (2nd extraction). The sticky extract was diluted required concentrations.

Table 1. List of microorganisms used

Gram positive bacteria	*Bacillus cereus* ATCC 27348
	Bacillus subtilis ATCC 9372
	Bacillus natto IFO 3009
	Streptococcus faecalis IFO 3971
	Staphylococcus aureus ATCC 13301
Gram negative bacteria	*Escherichia coli* ATCC 15489
	Salmonella typhimurium ATCC 14028
	Pseudomonas fluorescens ATCC 11250
Lactic acid bacteria	*Lactobacillus plantarum* ATCC 8014
	Lactobacillus brevis IFO 13110
	Luconostoc mesenteroides IFO 12060
	Pediococcus cerevisiae ATCC 11250
Yeast	*Saccharomyces cerevisiae* IFO 1950
	Hansenula anomala KCCM 11473
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Ethanol extract

The 1kg of leaf mustard was added to 3 ℓ ethanol, and the mixture was kept at room temperature. After 24 hrs, each extracts was filtered on whatman No. 2 (1st extraction). Six liter ethanol was added to the 1st extract and then treated by same method as 1st extraction. The extracts was evaporated in 50℃ water bath until to be 100 mℓ. One liter of distilled water was added to the above extracts and mixed well, put in 5℃ refrigerator. After 24 hrs, the extracts was centrifuged at 3,500 rpm twice to remove resin and then evaporated.

Antimicrobial activity measurement

Each strain was grown in a nutrient broth at 30℃ for 18-24 hrs prior to testing, and subcultured three times for 18-24 hrs. The turbidity of the bacterial cell suspensions was adjusted with the same sterile broth to a 0.3 optical density (OD) unit at 660 nm. The suspensions were then used for the tests. For the Disc plate method, 0.1mℓ of the bacterial cell suspension was poured uniformly into the plate. The paper discs containing the extracts were carefully placed on the seeded pertri dishes. The diameter of the inhibition zone was measured in millimeters after incubation at 30 ℃ for 24-48 hrs depending on the strains (Bauer *et al.*, 1966; Branch et al., 1965; Piddock, 1990). For the minimum inhibitory concentration (MIC) determination, the broth dilution method was used. MIC was determined as the lowest concentration that completely inhibited bacterial growth (MacLowry and Jaqua, 1970, Lee *et al.*, 1989).

Heat and pH stability of antimicrobial substrance

To evaluate the heat stability of the antimicrobial substances extracted from mustard the ethanol extract was heated at every 10℃ (60-100℃) for 1 hr and 121℃ for 15 minutes. And other procedures were accomplished as the method of agar diffusion. The diameter of inhibition zone was measured. For the experiment of pH stability, the ethanol extract was adjusted to pH 1~13 with HCl or NaOH and the extract was keep at room temperature for 1 hr. And then it was adjusted to optimal pH for microbes tested. And other procedures were accomplished as above heat stability experiments.

RESULTS AND DISCUSSION

Antimicrobial activity of Korean traditional leaf mustard extracts

Table 2 shows the results of antimicrobial activities and the MIC of leaf mustard ethanol extract. The extract was shown to have antimicrobial effects on most pathogens, yeasts, and lactic acid bacteria used for the study. Gram positive bacteria showed a higher sensitivity to the antimicrobial effect compared with gram negative bacteria. This result was similar to the results reported by Lee and Shin(1991) who examined natural antimicrobial substances inhibiting food spoilage microorganisms; by Hong *et al.*(1990) on *Ulmus pumila* L.; Park *et al.* (1992) who examined antimicrobial effects of herb medicine extracts; and Chung *et al.* (1990) who examined the antimicrobial effect of a substance purified from curry spice.

The miniumn inhibitory concenration(MIC) of ethanol leaf mustard extract

The results of miniumn inhibitory concentration of ethanol leaf mustard extracts are presented in Table 3. In this study, the MIC of the ethanol extract was lowest in *B. subtilis* and *B. natto* at 10 mg/mℓ and was between 15 to 20 mg/mℓ in other microbes, indicating that the antimicrobial effect was stronger in gram positive bacteria compared with gram negative bacteria. On the other hand, the MIC of lactic acid bacteria was relatively high at 40 mg/mℓ.

The antimicrobial effects of leaf mustard extract are relatively weak compared with the results of the study

by Lee *et al.* (1992) who reported that the MIC in 5 different gram positive bacteria including *B. subtilis*, and 5 different gram negative bacteria of *Ulmus pumila* L. extract, was between 2.5~30 ㎎/㎖. Even so, the antimicrovial activities of the leaf mustard extract that was examined in this study are significant. Ueda *et al.* (1982) found that many spices, including some of which used herein, were more inhibitory to gram positive bacteria than gram negative. Also Shelef *et al.*(1980) reported that gram positive bacteria were more sensitive to the spice sage, resemary and allspice than gram negative, and that coagolase-positive strains of *S. aureus* were paticularly sensitive. Farag *et al.* (1989) have reported that the major components of essential oils in some spices show MIC equal to that obtained with essential oils against some molds, bacteria and yeasts.

Heat and pH stability of antimicrobial substances

The results of heat stability of ethanol leaf mustard extract are presented in Table 4. The growth inhibition diameter of the temperature treatments to *B. cereus* (gram positive) and *E. coli* (gram negative) did not show the difference with control. It showed that the antimicorbial substances of the ethanol extract is stable to heat. Table 5 shows the pH stability of the antibacterial substances extracts from leaf mstard. It presents no changes antimicrobial activity in according to pH.

Table 2. Antimicrobial activities of water and ethanol extracts of Korean traditional leaf mustard

Strains	Clear zone on plate (mm)[a] (1.5 mg/disc)	
	Ethanol extract	Water extract
B. cereus	17	10
B. subtilis	22	11
B. natto	19	11
S. faecalis	14	10
S. aureus	19	10
L. plantarum	13	10
L .brevis	14	13
L. mesenteroides	16	12
P. cerevisiae	10	-[b]
E. coli	10	-
S. typhimurium	10	-
P. fluorescens	11	-
S. cerevisiae	10	11
S. coreanus	10	10
H. anomala	9	11

a) In diameter(mm), b) Not detected.

Table 3. Minimum inhibitory concentration (MIC) of the ethanol extracts against several microorganisms

Strains	MIC (mg/mℓ)	Strains	MIC (mg/mℓ)
B. cereus	15	*P. cerevisiae*	20
B. subtilis	10	*E. coli*	20
B. natto	10	*S. typhimurium*	20
S. faecalis	10	*P. fluorescens*	20
S. aureus	20	*S. cerevisiae*	60
L. plantarum	40	*S. coreanus*	40
L .brevis	40	*H. anomala*	40
L. mesenteroides	40		

Table 4. Effect of heat treatment on the growth inhibitory activity of ethanol extract for *B. cereus* and *E. coli*

Strains	Clear zone on plate (mm)[a] (1.5 mg/disc)							
	Heating temperature (℃)							
	Control	50	60	70	80	90	100	121
B. cereus	16.0	15.5	16.0	14.5	15.5	16.0	15.5	15.0
E. coli	14.0	14.0	14.0	13.5	14.0	14.0	13.5	13.5

a) diameter

Ethanol extract was heated for 60 min at 50 ~ 100 ℃ and heated for 15 min at 121 ℃.

Table 5. Effect of pH treatment on the growth inhibitory activity of ethanol extract for *B. cereus* and *E. coli*

Strains	Clear zone on plate (mm)[a] (1.5 mg/disc)							
	pH							
	Control	1	3	5	7	9	11	13
B. cereus	16.0	15.5	15.5	15.0	16.0	15.0	15.5	15.0
E. coli	14.0	14.5	14.5	14.5	14.5	14.0	14.0	14.0

a) diameter

The ethanol extract was adjusted to pH 1 ~ 13 for 60 min at room temperature.

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