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Bactericidal activity of wasabi (*Wasabia japonica*) against *Helicobacter pylori*

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Abstract

In this study, the bactericidal activity of Korean and Japanese wasabi roots, stems and leaves against *Helicobacter pylori* were examined. Allyl isothiocyanate (AIT) in roots, stems and leaves of Korean wasabi were 0.75, 0.18 and 0.32 mg/g, respectively. AIT in roots, stems and leaves of Japanese wasabi were 1.18, 0.41 and 0.38 mg/g, respectively. All parts of wasabi showed bactericidal activities against *H. pylori* strain NCTC 11637, YS 27 and YS 50. The leaves of both wasabi showed the highest bactericidal activities with the minimum bactericidal concentration of 1.05–1.31 mg of dry weight/ml against three strains of *H. pylori*. The roots showed a little lower bactericidal activity with 2.09–4.17 mg of dry weight/ml against them. The main component related to antimicrobial activity in wasabi is well known to be AIT. In this study, the bactericidal activity of leaves was higher than that of roots, although AIT amount of leaves was lower than that of roots. These results suggest that certain components besides AIT in wasabi are effective in killing *H. pylori*.

Keywords: Korean wasabi; Japanese wasabi; Allyl isothiocyanate (AIT); *Helicobacter pylori*; Minimum bactericidal concentration (MBC)

1. Introduction

Despite a marked decline in its incidence in many industrialized countries, cancer of the stomach remains the second most common cause of cancer-related deaths in the world, now exceeded only by lung cancer (Gowsala et al., 1997). The association between *Helicobacter pylori* infection and upper gastrointestinal diseases, such as chronic gastritis, peptic ulcer and gastric cancer, has been investigated (Warren, 1983; Marshall and Warren, 1984; Parsonnet et al., 1991). The eradication of *H. pylori* is seen as important in accelerating the healing and preventing the relapse of peptic ulcers (Rauws and Tytgat, 1990; Graham et al., 1991). However, the eradication is difficult, and the clinical trials with antibiotics alone have mostly failed to eradicate *H. pylori* (Mertens et al., 1989; Chiba et al., 1992). For example, amoxycillin treatment resulted in long-term *H. pylori* eliminates in only 20% of patients, although it is an effective agent for therapy of *H. pylori* infections (Barberis et al., 1989). In recent years, the increasing resistance of *H. pylori* to antibiotics further complicated the search for an optimal treatment (Ling et al., 1996; Midolo et al., 1996; Matsumoto et al., 1997).

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Therefore, a non-antibiotic agent that is both highly effective and safe could be important for the inactivation of *H. pylori*.

Many studies have been made on the antimicrobial activity of Japanese wasabi (*Wasabia japonica*). It has been reported that the essential oil of Japanese wasabi has a particularly strong antimicrobial effect against *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa* and other pathogenic bacteria (Nishida, 1958; Inoue et al., 1983). One of its components, allyl isothiocyanate (AIT), is mainly responsible for this bactericidal action (Foter and Golick, 1938; Forter, 1940; Isshiki et al., 1992). Hasegawa et al. (1999) also reported that wasabi or AIT inhibited the growth of *Vibrio parahaemolyticus*. Thus, it seems reasonable to explore the possibility of using the Wasabi for eradication of *H. pylori*.

In this study, we investigated the bactericidal activity of Korean and Japanese Wasabi against *H. pylori* and food-borne pathogenic bacteria in vitro.

2. Materials and methods

2.1. Materials

Korean wasabi (*W. japonica*) was obtained from a Korean wasabi farmer, who has been cultivating it at a spring-fed limpid stream in a forest in Chulwon, Kangwondo, Korea. Japanese wasabi (*W. japonica*) was obtained from a wasabi farmer in Tawaramine, Shizuoka, Japan. Both of the wasabi used in this study were harvested in the early spring after 2 years cultivation and stored at −80 °C. The standard allyl-, n-butyl-, 4-pentenyl-, 5-methylthiopentyl, 5-methylthiopentyl, 6-methylthiohexyl, 7-methylthioheptyl-, ethyl-, phenyl- and phenethyl isothiocyanate were purchased from Wako Pure Chemical Industries. (Osaka, Japan).

2.2. Preparation of the Wasabi extracts

Wasabi was separated into roots, stems and leaves parts, and washed thoroughly with distilled water. The extraction procedures with diethyl ether are described in Fig. 1. They were frozen with liquid nitrogen for 5 min and then pulverized with a blender. Two hundred grams of each part was mixed

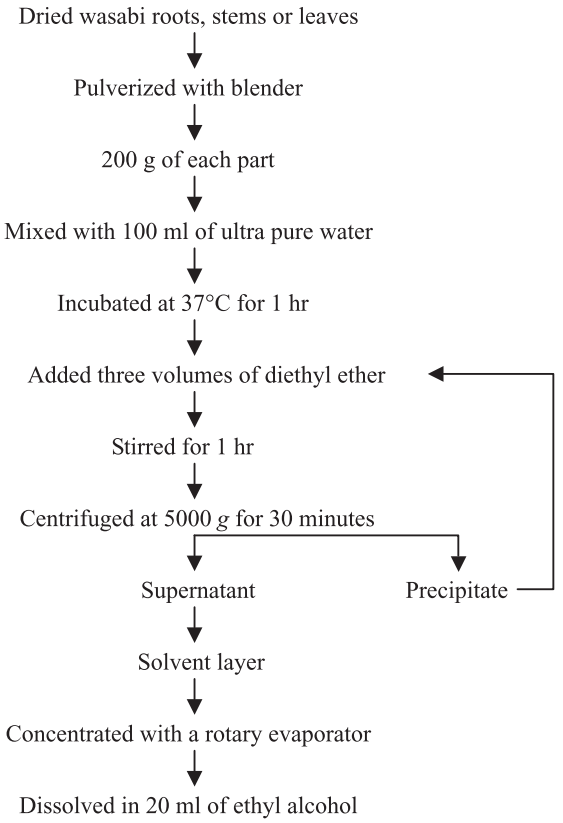

Fig. 1. Procedure for extraction of antibacterial components from wasabi.

with 100 ml of distilled water and agitated at 37 °C for an hour to maximize the yield of AIT. Three time-volume of diethyl ether was added to the mixture and stirred for an hour. The mixture was centrifuged at $5000 \times g$ for 30 min, and filtered through glass wool. The precipitates were extracted twice more with the same volume of diethyl ether. The supernatants pooled were separated into aqueous and solvent layers with a separating funnel. The solvent layer was concentrated with a rotary evaporator and the concentrate was dissolved in 20 ml of ethyl alcohol. The solution was applied to a Sep-Pak C_{18} cartridge (Waters, Milford, MA, USA) and sterilized by filtering through 0.22 μm filter of Millix-GS (Millipore, Bedford, MA, USA). Each sample solution contained 10 g of dry weight of root, stem or leaf per ml and was stored at −80 °C until use.

2.3. Quantitative analysis of AIT in Wasabi

The AIT concentrations in wasabi roots, stems and leaves were estimated by using a gas chromatograph (GL Sciences GC-380, Toronto, Canada) with a flame ionization detector (FID, Perkin-Elmer, Wellesley, MA, USA). A fused silica pillary column (0.25 mm i. d. × 30 m) was used. The oven temperature was programmed from 50 °C for 1 min to 200 °C for 3 min at 10 °C/min. The temperature of the injection port and the detector was kept at 220 °C. The carrier gas was helium, the split ratio being 1:50. The AIT and the other standard isothiocyanates were purchased from Wako Pure Chemical Industries. The Phenyl isothiocyanate (Wako Pure Chemical Industries) was used as an internal standard substance because of the absence in wasabi.

2.4. Culture of bacteria

H. pylori NCTC 11637 as reference strain, *H. pylori* YS 27 from a duodenal ulcer patient and *H. pylori* YS 50 from stomach cancer were obtained from Professor Ryushi Nozawa (University of Shizuoka, Japan). Fifty milliliters of Brucella broth (Difco Lab., Detroit, USA) with 0.5% of yeast extract, 5% of inactivated fetal bovine serum (BBL, Becton Dickinson, Cockeysville, MD) and 2% of vitox (Oxoid, Hampshire, England) was used for culture of *H. pylori* and for minimum bactericidal concentration (MBC) assay. *H. pylori* strains were cultured statically in an incubator with 5% CO_2 in air at 37 for 3 days. *E. coli* O157:H7 ATCC 43889, *V. parahaemolyticus* ATCC 2210001, *S. typhimurium* KCTC 058, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* KCTC 1012 and *Streptococcus mutans* KCTC 3298 were obtained from Korean Collection for Type Cultures (KCTC). The brain heart infusion broth (Difco Lab., Detroit, USA) was used for culture of them and for MBC assay.

2.5. Minimum bactericidal concentration assay for Korean and Japanese wasabi extracts

Minimum Bacterial Concentration (MBC) was assayed by the method of Bamba et al. (1997). Various concentrations of wasabi extract, the standard AIT and the other isothiocyanates were initially dissolved in ethyl alcohol and then serially diluted in a two-fold series with culture medium. Each bacterium was adjusted to 10^7 CFU/ml with culture medium, and 5 μl of the adjusted culture were inoculated in each well of the 96-well flat-bottom microplate (Nunc, Roskilde, Denmark), which had been filled with 100 μl of medium containing 50 μl of various concentrations of wasabi extraction. The plates were incubated in an incubator with 5% CO_2 in air at 37 °C for 3 days for *H. pylori* and in wide aerobic condition, i.e. in air in an incubator at 37 °C for 2 days for other bacteria. A loopful of each bacterial culture in each microplate well was inoculated to the culture medium agar plate, and incubated under the same conditions as described above. The MBC was defined as the lowest concentration that showed no colonies of bacteria on agar plates.

3. Results and discussion

3.1. AIT amount in wasabi

The main component related to antimicrobial activity in wasabi is well known to be AIT (Kanemura and Miyamoto, 1990; Isshiki et al., 1992; Nishida, 1958). We, therefore, measured the AIT amount in wasabi roots, stems and leaves. The retention time of AIT on a gas chromatogram was about 6.04–6.21 min (Fig. 2). The AIT amounts in Korean and Japanese wasabi roots, stems and leaves are shown in Table 1. The AIT amounts of Japanese wasabi appeared to be a little larger than those of Korean wasabi. The AIT amount was the highest in roots of both wasabi.

3.2. Minimum bactericidal concentrations of wasabi against H. pylori and food-borne pathogenic bacteria

The MBCs of Korean wasabi against three strains of *H. pylori* and food-borne pathogenic bacteria are shown in Table 2. All parts of Korean wasabi showed a bactericidal activity against all bacteria tested. Korean wasabi leaves showed the highest bactericidal activity with a MBC of 1.05–1.31 mg of dry weight/ml against *H. pylori* strains. The root showed a little lower bactericidal activity with a MBC of 2.61–4.17

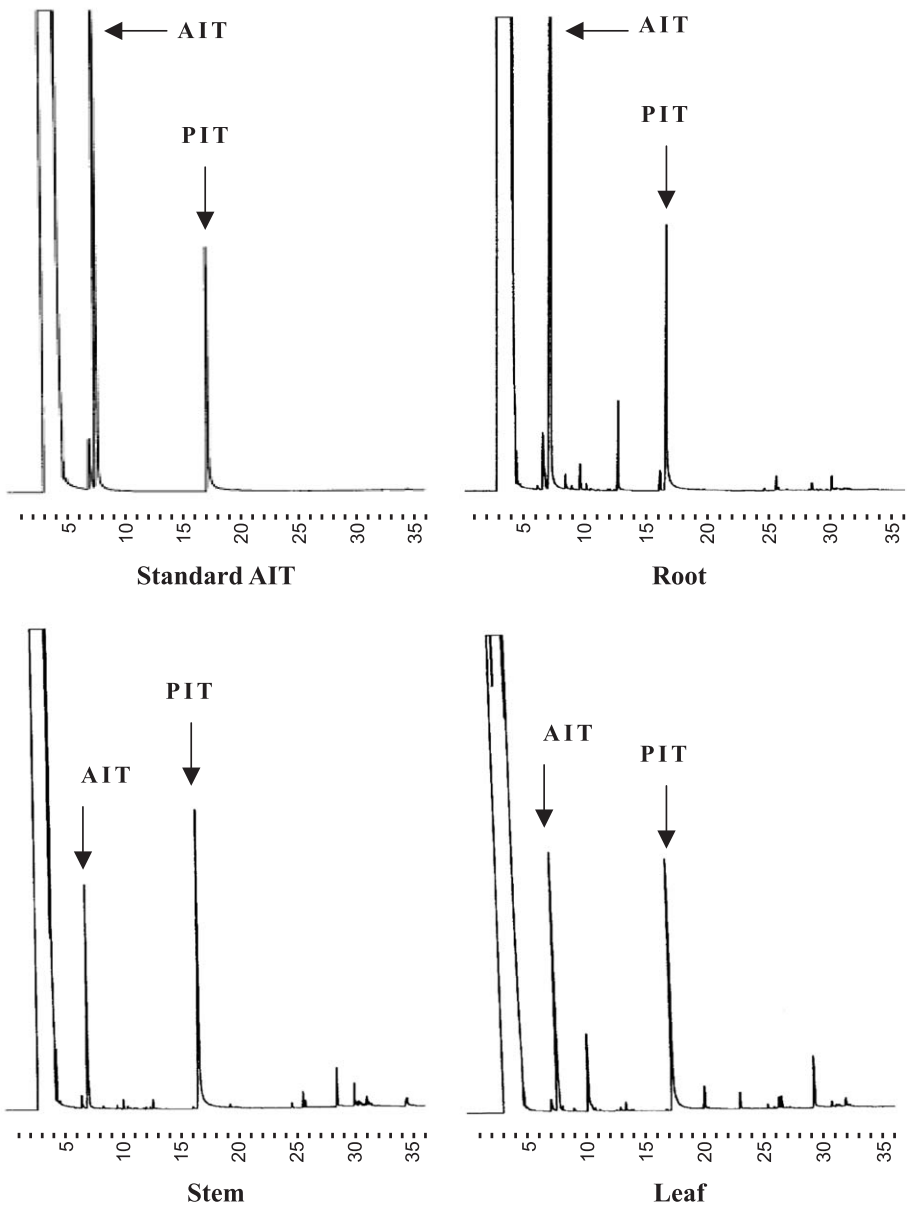

Fig. 2. Typical gas chromatogram of allyl isothiocyanate (AIT) in roots, stems and leaves of wasabi. PIT is peak of phenyl isothiocyanate added as an internal standard.

mg of dry weight/ml against them. The MBC values of Japanese wasabi against three strains of *H. pylori* are shown in Table 3. Japanese wasabi leaves showed the strongest bactericidal activity with MBC of 1.05–1.31 mg of dry weight/ml. Japanese wasabi roots (2.09–2.61 mg of dry weight/ml) showed just a little higher bactericidal activity than Korean wasabi against *H. pylori* strains. There were no significant differences in bactericidal activity between Korean wasabi and Japanese wasabi against *H. pylori*. We evaluated also the bactericidal activity of other isothiocyanates, which are known to occur in the wasabi

Table 1
Allyl isothiocyanate (AIT) amounts of wasabi roots, stems and leaves

Wasabi	AIT amount (mg/g)		
	Roots	Stems	Leaves
Korean wasabi	0.75	0.18	0.32
Japanese wasabi	1.18	0.41	0.38

against *H. pylori* (Table 4). The bactericidal activities (MBC) of most isothiocyanates such as 4-pentenyl (0.42–0.67 mg of dry weight /ml), 5-methylthiopentyl (0.25–0.33 mg of dry weight /ml), 6-ethylthiohexyl (0.21–0.25 mg of dry weight /ml), 7-methylthioheptyl (0.42–0.67 mg of dry weight /ml) and phenethyl isothiocyanates (0.13–0.17 mg of dry weight /ml) were stronger than that of AIT (1.33–2.67 mg of dry weight /ml). Katsuhiro et al. (1999) reported that the minimum inhibition concentration (MIC) of epigallocatechin gallate (EGCg) of green tea against *H. pylori* was 32 μg/ml and MBC was 128 μg/ml. They used purified EGCg purchased from Mitusi Norin (Shizuoka, Japan). The content of EGCg in green tea is in the order of 7.53% (dry weight).

The 32 μg/ml of EGCg is equal to 1.70 mg of dry weight of green tea. Korean and Japanese, usually consume about 1–3 g of wasabi when they eat sashimi (sliced row fish and shellfish) or sushi (vinegary rice ball topped with sliced row fish). Thus, we think that wasabi could play a role in *H. pylori*, in vivo.

In case of food-borne pathogenic bacteria, Korean and Japanese wasabi showed the highest bactericidal activity with a MBC of 0.27–0.66 mg of dry weight/ml against *V. parahaemolyticus* (Tables 2 and 3). Both wasabi showed higher bactericidal activity against Gram-negative bacteria (*E. coli* O157:H7, *V. parahaemolyticus* and *S. typhimurium*) than Gram-positive bacteria (*S. aureus*, *B. cereus* and *S. mutans*). The bactericidal activities of the both wasabi were higher against food-borne pathogenic bacteria than against *H. pylori*.

Wasabi is used as a spice to avoid both food poisoning and odor (fishy smell) of Japanese traditional foods such as sashimi (sliced row fish and shellfish) and sushi (vinegary rice ball topped with sliced row fish). AIT is a major pungent component of wasabi, black mustard and horseradish, and well

Table 2
Minimum bactericidal concentrations (MBC) Allyl isothiocyanate (AIT) from Korean wasabi against *Helicobacter pylori* and food-borne pathogenic bacteria

Tested strains	Standard AIT (99%, mg/ml)	Korean wasabi (mg of dry weight/ml)		
		Roots	Stems	Leaves
Helicobacter pylori NCTC 11637	0.67 ± 0.29[a]	2.61 ± 0.90	5.21 ± 1.80	1.05 ± 0.45
Helicobacter pylori YS 27	0.67 ± 0.29	2.61 ± 0.90	4.17 ± 1.80	1.05 ± 0.45
Helicobacter pylori YS 50	0.33 ± 0.11	4.17 ± 1.80	5.21 ± 1.80	1.31 ± 0.45
Escherichia coli O157:H7 ATCC 43889	0.67 ± 0.29	0.66 ± 0. 22	1.57 ± 0.90	0.33 ± 0.11
Vibrio parahaemolyticus ATCC 2210001	0.21 ± 0.07	0.33 ± 0.11	0.66 ± 0.22	0.27 ± 0.11
Salmonella typhimurium KCTC 2058	0.67 ± 0.29	1.31 ± 0.45	2.09 ± 0.90	0.66 ± 0.22
Staphylococcus aureus ATCC 25923	2.67 ± 1.15	5.21 ± 1.80	8.33 ± 3.60	4.17 ± 1.80
Bacillus cereus KCTC 1012	2.67 ± 1.15	4.17 ± 0.90	5.21 ± 1.80	4.17 ± 0.90
Streptococcus mutans KCTC 3298	1.67 ± 0.58	2.09 ± 0.90	2.61 ± 0.90	1.31 ± 0.45

[a] Means of three measurements ± standard deviation.

Table 3
Minimum bactericidal concentrations (MBC) Allyl isothiocyanate (AIT) from Japanese wasabi against *Helicobacter pylori* and food-borne pathogenic bacteria

Tested strains	Standard AIT (99%, mg/ml)	Japanese wasabi (mg of dry weight/ml)		
		Roots	Stems	Leaves
Helicobacter pylori NCTC 11637	1.33 ± 0.58[a]	2.09 ± 0.90	5.21 ± 1.80	1.05 ± 0.45
Helicobacter pylori YS 27	1.33 ± 0.58	2.61 ± 0.90	8.33 ± 3.60	1.31 ± 0.45
Helicobacter pylori YS 50	2.67 ± 1.15	2.09 ± 0.90	5.21 ± 1.80	1.31 ± 0.45
Escherichia coli O157:H7 ATCC 43889	0.67 ± 0.29	0.66 ± 0.22	2.09 ± 0.90	0.33 ± 0.11
Vibrio parahaemolyticus ATCC 2210001	0.21 ± 0.07	0.33 ± 0.11	0.66 ± 0.22	0.27 ± 0.11
Salmonella typhimurium KCTC 2058	0.67 ± 0.29	1.31 ± 0.45	1.57 ± 0.90	0.33 ± 0.11
Staphylococcus aureus ATCC 25923	5.33 ± 2.31	5.21 ± 1.80	10.4 ± 3.60	2.61 ± 0.90
Bacillus cereus KCTC 1012	2.67 ± 1.15	4.17 ± 0.90	5.21 ± 1.80	2.09 ± 0.90
Streptococcus mutans KCTC 3298	1.67 ± 0.58	2.09 ± 0.90	2.61 ± 0.90	1.31 ± 0.45

[a] Means of three measurements ± standard deviation.

known to have strong antimicrobial activity (Foter and Golick, 1938; Forter, 1940; Kanemura and Miyamoto, 1990; Isshiki et al., 1992). Many have studied AIT in order to fully utilize its bactericidal activity. Ogawa et al. (1998) have studied the bactericidal and bacteriostatic effects by hydrostatic pressure treatment upon addition of AIT. In the examination of growth of *S. aureus* and *Bacillus subtilis*, the lag phase of strains treated with 200 Mpa with addition of AIT was prolonged. Hasegawa et al. (1999) have demonstrated used 60 mg of wasabi/ml containing 67.932 μg of AIT/ml inhibited the growth of *V. parahaemolyticus* which is near to level of 101.7 μg of AIT/g in fish meat.

The results of the study suggest that wasabi contains certain components, such as phenol compounds, besides AIT with bactericidal activity against *H. pylori* and food-borne pathogenic bacteria. Although the components responsible for the bactericidal activity are not fully known, the purification of anti-*H.*

Table 4
Minimum bactericidal concentrations (MBC) of several isothiocyanates against three strains of *Helicobacter pylori*

Isothiocyanates		MBC (mg of dry weight/ml)		
		NCTC 11637	YS 27	YS 50
Allyl	CH_2CHCH_2NCS	1.33 ± 0.58[a]	1.33 ± 0.58	2.67 ± 1.15
n-Butyl	$CH_3(CH_2)_3NCS$	0.83 ± 0.29	1.00 ± 0.00	1.33 ± 0.58
4-Pentenyl	$CH_2CH(CH_2)_3NCS$	0.67 ± 0.29	0.42 ± 0.14	0.67 ± 0.29
5-Methylthiopentyl	$CH_3S(CH_2)_5NCS$	0.33 ± 0.14	0.33 ± 0.14	0.25 ± 0.00
6-Methylthiohexyl	$CH_3S(CH_2)_6NCS$	0.25 ± 0.00	0.25 ± 0.00	0.21 ± 0.07
7-Methylthioheptyl	$CH_3S(CH_2)_7NCS$	0.42 ± 0.14	0.42 ± 0.14	0.67 ± 0.29
Ethyl	C_2H_5NCS	0.83 ± 0.29	1.00 ± 0.00	1.33 ± 0.58
Phenyl	C_6H_5NCS	1.00 ± 0.00	0.83 ± 0.29	1.33 ± 0.58
Phenethyl	$C_6H_5CH_2CH_2NCS$	0.13 ± 0.00	0.17 ± 0.07	0.13 ± 0.00

[a] Means of three measurements ± standard deviation.

pylori components from the wasabi leaves are in progress to explore the full potential of the wasabi as a bactericidal agent against *H. pylori*.

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