Food Sci. Technol. Res., **14** (2), 190–193, 2008

Note

Screening of Antibacterial Activities of Edible Plants against *Streptococcus mutans*

Akihiro OHARA*, Fumie SAITO and Tsugio MATSUHISA

Graduate School of Agricultural Science, Meijo University, Nagoya 468-8502, Japan

Received December 6, 2005; Accepted October 13, 2007

The antibacterial activities of 81 edible plants against the dental caries pathogen *Streptococcus mutans* were investigated. The fresh vegetative crude extracts were subjected to the paper disc method. Furthermore, in order to fractionate the active component, hexane, ethyl acetate and methanol extracts from freeze-dried samples were also examined. Antibacterial activities were positive in 17 samples, including cinnamon and Japanese ginger. Among these, the stabilities of the active components against heat treatment or storage at 4 ℃ for one week were also investigated. Following these treatments, the activities of balsam pear and garlic extracts were lost, while the active components in ginger, Japanese ginger, clove and cinnamon appeared. Samples of the genus *Zingiberaceae*, including Japanese ginger and ginger, contained abundant and stable antibacterial components acting against *S. mutans*.

Keywords: *Streptococcus mutans*, antibacterial activity, paper disc, ginger, Japanese ginger

Introduction

Recently, many phytochemicals, including antibacterial agents, have been clarified from edible plants (Hirasawa *et al.*,1999; Seo et al., 2001; Jayaprakasha *et al.*, 2003). There are also numerous reports on the components of plants, which have revealed antibacterial activities against *Streptococcus mutans* (*S. mutans*), which is widely known as a cause of dental caries (Brady *et al.*, 2002; 2003; Yamada *et al.*,1999; Takeuchi *et al.*, 2000). This strain is detected in all human populations, regardless of culture, eating habits, and life-style (Kozai *et al.*, 1999). In an aged society, such as Japan, it is useful to lower the threat of lifestyle-related diseases by preventing dental caries. Dental health is in fact one of the improvement items in "Health Japan 21", which is a campaign by the Ministry of Health, Labour and Welfare of Japan (Ministry of Health, Labour and Welfare, 2004).

From the viewpoint of health maintenance, it is important to clarify effective agents against this bacteria derived from natural materials. Therefore, we screened effective, natural and safe food factors for controlling *S. mutans*.

Materials and Methods

Chemicals Bacto Brain Heart Infusion (BHI) was a product of Oxoid Ltd., (Basingstoke, UK). Solvents for extracting active components from samples were purchased from Wako Pure Chemical Ind., Ltd., Tokyo, Japan.

Microorganisms *Streptococcus mutans* NBRC 13955 (*S. mutans*) was provided by the National Institute of Technology and Evaluation Biological Resource Center (Kisarazu, Japan). The bacterium was pre-cultured overnight at 37 °C in BHI broth (5 mL). This bacterial suspension was diluted with BHI broth and used for measuring antibacterial activities.

Extraction from edible plant material The 72 edible plants examined were purchased from a market in Nagoya City, Japan. Nine kinds of micro algae, including chlorella and spirulina, were provided by the Micro Algae Corporation (Gifu, Japan). The scientific, common and Japanese names of these samples are listed in Table 1. Each freeze-dried sample was extracted with 10 volumes of several different solvents for 20 minutes, and then each resulting extract was evaporated to dryness. The solvents used were hexane, ethyl acetate and methanol, in order of polarity. After extracting with the first solvent three times, the residue was successively extracted with the next solvent. Each resulting extract was dissolved at 100 mg/mL in dimethyl sulfoxide (DMSO) and submitted to the bioassay for antibacterial activity. In addition, a crude extract, which freeze-dried sample was extracted by water, was also tested.

Determination of antibacterial activity Antibacterial

*To whom correspondence should be addressed.
E-mail: oharaaki@ccmfs.meijo-u.ac.jp

Table 1. List of 81 examined samples.

Family	Common Name (Japanese Name)			
Apiaceae	Carrot	Caraway	Dill (Leaf)	Dill (Seed)
	Japanese honewort	Cumin	Fennel	Parsley
	Celery			
Araceae	Taro (Sato-imo)	Taro (Ise-imo)	Taro (Serubesu)	
Asteraceae	Edible burdock	Garland chrysanthemum	Gynura	
Brassicae	Broccoli	Cabbage	Chinese cabbage	Turnip (Kabu)
	Turnip (Aka-Kabu)	Japanese radish	Radish (Shogoin)	Radish (Akamaru)
	Radish (Karami-Daikon)	Wasabi	Sprout of Japanese radish	
Chenopodiaceae	Garden-beet	Spinach		
Convolvulaceae	Sweet potato	Sweet potato (purple)	Balsam pear	
Cucurbitaceae	Pumpkin			
Dioscoreaceae	Yam			
Fabaceae	Peanut	Green soybean	Bean sprout	Field snap bean
	Garden pea			
Gramineae	Corn			
Illicium	Star anise			
Labiatae	Perilla (O-oba)	Perilla (Aka-shiso)	Thyme	
Lauraceae	Cinnamon	Laurel		
Liliaceae	Onion	Onion (Pekorosu)	Red onion	Shallot
	Belgian shallot	Garlic	Stem of garlic	Chinese chive
	Aloe	Bulb of lily	Asparagus	
Malvaceae	Okra			
Myristicaceae	Nutmeg			
Myrtaceae	Clove	Allspice		
Nymphaeaceae	East Indian lotus			
Piperaceae	Pepper (white)	Pepper (black)		
Rubiaceae	Gardenia			
Rutaceae	Japanese pepper			
Solanaceae	Red pepper	Sweet pepper	Sweet pepper (Shishito)	Tomato
	Potato			
Tiliaceae	Jew's mallow			
Zingiberaceae	Japanese ginger	Ginger		
Micro Algae	Chlorella	Dunaliella	Nostoc c.	Nostoc fla.
	Peragophysia sp	Pleurochrysis	Porphyridium	Rhodsorus
	Spirulina			

activity was measured by an agar diffusion method (Abe *et al.*, 2004). Pre-cultured bacterial suspension (*S. mutans*) was diluted to A_{660} 0.12 with BHI broth. This diluted suspension contained the viable bacteria at 10^7 cells/mL. Two hundred micro liters of diluted bacterial suspension was stratified on BHI agar medium, and the 15 μL sample solution was applied to a paper disc. The plate was refrigerated overnight, and then cultivated for 24 hours at 37 °C. The activity was evaluated at the diameter of the inhibition zone, occurring at the circumference of the paper disc.

Results and Discussion

The antibacterial activities using the paper disc method for 81 edible plants (55 vegetables, 17 spices, and 9 micro algae) against *S. mutans* were tested (Table 1). If an inhibition zone over 6 mm in diameter was observed for the sample (100 mg/mL) applied paper disc, its activity was considered positive. Cases in which the inhibition zone was not formed were judged to be negative.

Of the crude extracts (juices), 4 samples, garlic (bulb, 30.0 mm), onion (6.0 mm), Belgian shallot (6.0 mm) and balsam pear (6.0 mm), exhibited antibacterial activities. In this experiment, most of the samples showing antibacterial activities were bulbs belonging to the *Allium* genus.

However, in extracts from polarity-differing solvents, 17 edible plants showed antibacterial activities at least in one fraction. Of the hexane extracts, the 12 samples which showed antibacterial activities were cinnamon (18.5 mm, 20 mg/g), Japanese ginger (9.7 mm, 8 mg/g), ginger (8.8 mm, 23 mg/g), balsam pear (8.0 mm, 4 mg/g), clove (6.4 mm, 210 mg/g), wasabi (6.3 mm, 8 mg/g), thyme (6.0 mm, 69 mg/g), garlic (bulb, 6.0 mm, 6 mg/g), onion (6.0 mm, 7 mg/g), red

onion (6.3 mm, 5 mg/g), Belgian shallot (6.0 mm, 6 mg/g), and Japanese pepper (6.0 mm). Of the ethyl acetate extracts, antibacterial activities were found in ginger (8.3 mm, 8 mg/g), Japanese ginger (8.2 mm, 6 mg/g), thyme (7.7 mm, 28 mg/g), garlic (bulb, 7.0 mm, 0.3 mg/g), Belgian shallot (6.0 mm, 3 mg/g), perilla (6.0 mm, 30 mg/g), nutmeg (6.2 mm), rhodsorus (6.0 mm), and Japanese pepper (6.0 mm). However, extracts that showed activities in the high polarity solvent, methanol, only numbered 4: clove (8.7 mm), beets (6.0 mm), chlorella (6.0 mm), and dunaliella (6.0 mm). From these data, it was indicated that many antibacterial components acting against *S. mutans* in edible plants are low-polarity materials. This result is different from previous reports that the active components from edible plants are water-soluble polyphenolic components (Taguri *et al.*, 2004; Smullen *et al.*, 2007).

Thyme, garlic, Belgian shallot, Japanese pepper, Japanese ginger and ginger showed activity in the extracts of hexane and ethyl acetate indicating that some active components were included in these samples. Only the hexane extract of cinnamon showed high activity. Additionally, one of the highest antibacterial samples was Japanese ginger. The antibacterial components of Japanese ginger against food-poisoning bacteria have already been reported and identified (Abe *et al.*, 2004; 2002), and, as shown in the present experimental results, all of its components are soluble in ethyl acetate.

The relative activities of samples which showed the antibacterial activities were calculated from the concentration of ampicillin, which is equal to the diameter of the samples showing an inhibition circle. The inhibition zone shown by the hexane extract from cinnamon (100 mg/mL) corresponds to 100 μg/mL of the ampicillin concentration. Therefore, the relative activity of cinnamon's hexane extract was 1/1000 of ampicillin. Relative activity of the ethyl acetate extract from ginger and the methanol extract from clove was 1/5,000 of ampicillin, and 1/125 in the crude garlic extract, which showed the highest antibacterial activity. As in a previous report (Elgayyar *et al.*, 2001), the activities of the extracts from edible plants were not higher than antibiotics.

There are few opportunities to eat edible plants soon after harvesting, as they are often stored in a refrigerator before consumption and usually eaten after heat treatment. Therefore, the stability of the active components from samples that showed high activities was examined after temperature treatments (Table 2).

The stability of antibacterial components from highly active samples was examined after refrigeration (4 °C) for one week. Garlic and balsam pear were inactivated, but the activities of other samples (clove, nutmeg, thyme, ginger, Japanese ginger, and cinnamon) were maintained.

In addition, the changes of activity following heat treatment at 100 °C for 10 minutes were also examined. The activities of garlic and balsam pear disappeared, and that of cinnamon was lowered. However, the activities of clove, nutmeg, thyme, ginger and Japanese ginger were unchanged.

Generally, most of the samples which showed activities in this experiment were herbs and spices. Furthermore, the active components from these samples were also stable following several storage processes. Past reports that "herbs and spices" generally showed antibacterial activities against food-poisoning bacteria such as *Salmonella* are frequent (Odate *et al.*, 2000). It was also reported that polyphenolic compounds in spices (high-polarity fraction) showed inhibitory effects against dental caries bacteria (Hattori *et al.*, 1986). In the present experiment, low-polarity extracts of several spices from the *Labiatae*, *Liliaceae*, and *Zingiberaceae* families showed activities against *S. mutans*. Moreover, the antibacterial activity of the ginger family, such as Japanese ginger, was both stable and high; the extracted volume from 1 g of sample was over 10 mg. In the future, it will be important to clarify this active component.

References

Abe, M., Ozawa, Y., Uda, Y., Yamada, F., Morimitsu, Y., Nakamura, Y. and Osawa, T. (2004). Antimicrobial activities of diterpene dialdehydes, constituents from Japanese ginger (*Zingiber mioga Roscoe*), and their quantitative analysis. *Biosci. Biotechnol. Biochem.*, **68**, 1601-1604.

Abe, M., Ozawa, Y., Uda, Y., Yamada, F., Morimitsu, Y., Nakamura,

Table 2. The physicochemical properties of active samples.

	Fresh			After boiling for 10 min.			After storage for one week at 4 ℃		
	Hexane	Ethyl acetate	Methanol	Hexane	Ethyl acetate	Methanol	Hexane	Ethyl acetate	Methanol
Japanese ginger (*Zingiber mioga* ROSC.)	9	9	0	9	8	0	9	7	0
Ginger (*Zingiber officinale* ROSCOE.)	9	8	0	8	7	0	8	7	0
Garlic (*Allium sativum* L.)	7	7	0	0	0	0	0	0	0
Balsam pear (*Momordica charantia* L.)	7	0	0	0	0	0	0	0	0
Thyme (*Thymus vulgaris* L.)	6	8	0	6	8	0	6	8	0
Nutmeg (*Myritica fragrans* Houtt.)	0	6	0	0	6	0	0	8	0
Cinnamon (*Cinamonum verum* J. Presl)	19	0	0	14	0	0	8	0	0
Clove (*Syzygium aromaticum* L.)	6	0	9	6	0	9	7	0	9

The activity was showed the diameter of inhibition zone (mm). Changes in antibacterial activities against *S. mutans* by heat treatment (boiling for 10 min) and temperature storage (4 °C) were measured. Samples showed antibacterial acti vity for either fraction.

Y. and Osawa, T. (2002). Labdane-type diterpene dialdehyde, pungent principle of Japanese ginger, *Zingiber mioga Roscoe*. *Biosci. Biotechnol. Biochem.*, **66**, 2698-2700.

Brady, D., Gaines, S., Fenelon, L., Mcpartlin, J. and O'Farrelly, C. (2002). A Lipoprotein-derived antimicrobial factor from hen-egg yolk is active against *streptococcus* species. *J. Food Sci.*, **67**, 3096-3103.

Brady, D., Lowe, N., Gaines, S., Fenelon, L., Mcpartlin, J. and O' Farrelly, C. (2003). Inhibition of *Streptococcus mutans* growth by hen egg-derived fatty acids. *J. Food Sci.*, **68**, 1433-1437.

Elgayyar, M., Draughon, F. A., Golden, D. A. and Mount, J. R. (2001). Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. *J. of Food Protect.*, **64**(7), 1019-1024.

Hattori, M., Hada, S., Watahiki, A., Ihara, H., Shu, Y-Z., Kakiuchi, N., Namba, T. and Mizuno, T. (1986). Studies on dental caries prevention by traditional medicines. X Antibacterial action of phenolic components from mace against *Streptococcus mutans*. *Chem. Pharm. Bull.*, **34**, 3885-3893.

Hirasawa, M., Shouji, N., Neta, T., Fukushima, K. and Takeda, K. (1999). Three kinds of antibacterial components from Lentinus edodes (Berk.) Sing. *Int. J. Antimicrob. Agents*, **11**, 151-157.

Jayaprakasha, G. K., Selve, Tamil and Sakariah, K. K. (2003). Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts. *Food Research International.*, **36**, 117-122.

Kozai, K., Nakayama, R., Tedjosasongko, U., Kuwahara, S., Suzuki, J., Okada, M. and Nagasaka, N. (1999). Intrafamilial distribution of *mutans* streptococci in Japanese families and possibility of father-to-child transmission. *Microbiol. Immunol.*, **43**, 99-106.

Odate, J., Yamada, K. and Ishii, E. (2000). Spice treatment of chicken and its antibacterial activity (in Japanese). *J. Cookery Sci. Jpn.*, **33**, 358-364.

Seo, K. I., Moon, Y. H., Choi, S. U. and Park, K. H. (2001). Antibacterial activity of S-methyl methanethiosulfinate and S-methyl 2-propene-1-thiosulfinate from chinese chive toward *Escherichia coli* O157:H7. *Biosci. Biotechnol. Biochem.*, **65**, 966-968.

Smullen, J., Koutsou, G. A., Foster, H. A., Zumbe, A. and Storey, D. M. (2007). The Antibacterial Activity of Plant Extracts Containing Polyphenols against *Streptococcus mutans*. *Caries Research*, **41**, 342-349.

Taguri, T., Tanaka, T. and Kouno, I. (2004). Antimicrobial activity of 10 different plant polyphenols against bacteria causing food-borne disease. *Biol. Pharm. Bull.*, **27**, 1965-1969.

Takeuchi, H., Senpuku, H., Matin, K., Kaneko, N., Yusa, N., Hanada, N., Yoshikawa, E., Kono, Y. and Toyoshima, Y. (2000). New dental drug delivery system for removing *mutans Streptococci* from the oral cavity. Effect on oral microbial flora. *Jpn. J. Infect Dis.*, **53**, 211-212.

Ministry of Health, Labour and Welfare (2004). The White Book on Health, Labour and Welfare, Gyosei Ltd., Tokyo, Japan, 66-75.

Yamada, Y., Yamamoto, A., Yoneda, N. and Nakatani, N. (1999). Identification of kaempferol from the leaves of *Diospyros kaki* and its antimicrobial activity against *Streptococcus mutans*. *Biocontrol Sci.*, **4**, 97-100.