Note

Anti-Tumor Promoting Activities of Edible Plants against Okadaic Acid

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The anti-tumor promoting activities of 120 kinds of edible plants against the non TPA-type promoter, okadaic acid (OA), were investigated by measuring suppression of the inhibitory effect of OA on protein phosphatase-2A (PP2A), since OA promotes tumor formation by inhibiting this enzyme. One quarter of the tested samples showed activity over 20%. In particular, 8 kinds of plants (basil (100%), peppermint (100%), carrot leaf (100%), broccoli (100%), nabana (98%), edible burdock (86%), watermelon (99%) and winter fungus (100%)) showed very strong activity. Physicochemical properties of the active components contained in the samples that showed high activity were found that the active component in the major sample was inactivated by cold storage or boiling, and that only edible burdock showed strong activity following both types of processing. Soluble fractions from these samples were obtained by extraction in hexane, benzene, ethyl acetate, methanol and water. Very strong anti-OA activity was observed in water soluble extracts of edible burdock and eggplant, whereas cabbage and broccoli showed activity in the extraction fractions in organic solvents such as hexane. Although the active components in these samples were not identified, these findings suggest that the component with anti-okadaic acid activity must be present in edible plants.

Keywords: okadaic acid, anti-tumor promoting activity, protein phosphatase-2A (PP2A), edible plants, edible burdock

Seventy percent of the causes of carcinogenesis are related to food and smoking (Nakamura *et al.*, 1990). Carcinogens derived from smoking can be avoided by stopping the habit of smoking; however it is difficult to avoid the carcinogens consumed in daily foods. It is known that many carcinogens and/or mutagens, such as heterocyclic amines (Sugimura, 1995) or *N*-nitroso compounds (Ohara *et al.*, 1988), are present in food, although evidence that some foods actually prevent cancer has also been reported (Osawa, 1995, 1999; Watanabe *et al.*, 1997).

Cancer develops through a multistep process (Sugimura, 1982). The first stage of carcinogenesis is caused by chemical compounds called initiators. The initiation signifies a gene mutation, and a normal cell becomes a precancerous cell. Most mutagens are also carcinogens. Therefore, antimutagenic properties are sought in various compounds as potential inhibitors of initiations, and much information on this has been accumulated (Natake *et al.*, 1989; Kanazawa *et al.*, 1995; Samejima *et al.*, 1995; Ohara & Hiramatsu, 1997; Ohara & Takehara, 1997; Ohara *et al.*, 2000).

The second stage of cancer development is promotion, in which the precancerous cell becomes a cancer cell. This stage is a reversible phenomenon, however, and it is very important to suppress this step to prevent cancer. Promotion is caused by a promoter compound such as TPA (12-*O*-tetradecanoylphorbol-13-acetate), but there are many reports of anti-tumor promoting activity against TPA (Koshimizu, 1995, 1996; Ohigashi *et al.*, 1997). There are, however, many promoters which operate via different mechanisms. One of them is okadaic acid (OA) which is also known as a diarrhetic shellfish poison, and as a PP2A (protein phosphatase type-2A) inhibitor (Fujiki, 1992). OA accelerates carcinogenesis in the liver and many other organs,

while TPA promotes only cutaneous cancer (Matias *et al.*, 1999; Fujiki & Suganuma, 1999; Traore *et al.*, 2000). There is thus a strong possibility of suppressing the promotion process of carcinogenesis on the material which inhibits the OA. There have been only a few reports on OA (Murata *et al.*, 1982; Suganuma & Fujiki, 1993).

Here, we searched for anti-OA activity in 120 edible plants, and examined all properties of the active ingredients.

Materials and Methods

Materials The 120 edible plants examined were general commercial products. Okadaic acid was purchased from Wako Pure Chemical Ind. Ltd. *p*-Nitro phenylphosphate (*p*-NPP) was purchased from Sigma-Aldrich Fine Chemicals (USA), and PP2A was from Upstate Biotechnology (USA). All other chemicals were of high grade.

Extracts from edible plants The water extract from each sample was obtained with a juice mixer. The sample solution was filtered to eliminate bacteria, then it was used for the experiment on anti-OA activity.

Anti-OA activity PP2A is inhibited by OA, and since the substrate specificity is low, various phosphates are hydrolyzed by PP2A. Based on this property, a simple testing method was established for PP2A in which *p*-NPP was the substrate (Takai & Mieskes, 1991, Takai *et al.*, 1992). Using this method, Ogino *et al.* (1997) proposed a method of measuring anti-OA. On samples which inhibited the tumor promoter, OA, a strong possibility was found of preventing the cancer. The inhibition ratio of the sample solution against okadaic acid was measured against an index.

Unless otherwise stated, PP2A was diluted with a buffer containing 40 mM-Tris/HCl, 20 mM-KCl, 30 mM-MgCl₂ and 2 mM-

DL-dithiothreitol (pH 8.1) and 1 mg of BSA/ml. Okadaic acid was dissolved in dimethyl sulfoxide (DMSO) to give a 10 mM solution and diluted in this buffer. The maximal concentration of DMSO in the reaction mixture was 0.01% (v/v). Control activities were not significantly affected by addition of this amount of DMSO.

The sample solution was incubated with an OA solution for 20 min (reaction mixture). Thereafter, to assay anti-OA activity, reaction was started by adding PP2A to a micro tube containing *p*-NPP and the reaction mixture. The sample was incubated for 60 min and absorbance of the mixture was measured at 405 nm. All procedures were carried out at 30°C.

By deducting the amount absorbed by the sample solution, the effect of the absorption by the sample was excluded. Also, on the samples which showed anti-OA activity, it was determined whether or not the similar action with PP2A existed.

Anti-OA activity was classified into 4 levels by comparison with the control:

Very strong (++): Enzyme inhibition of OA suppressed over 80%.

Strong (+): OA suppressed from 40% to 79%.

Weak (±): OA suppressed from 20% to 39%.

None (–): OA suppressed to below than 19%.

Results and Discussion

Anti-OA activity The anti-OA activity in the water extracts of 120 edible plants was measured (Table 1). Eight types (7%) of plants showed very strong activity (++) with over an 80% inhibition ratio against OA. Specifically, these sample solutions showed the following activities: basil (100%), peppermint (100%), carrot leaf (100%), broccoli (100%), nabana (98%), edible burdock (86%), watermelon (99%) and winter fungus (100%). Sixteen types (14%) of tested plants including turnip (47%), cauliflower (67%), agar-agar (41%), cherry (58%), bitter

gourd (43%) and chestnuts (51%) showed strong activity (+) of less than 80% but over 40% inhibition ratio, and eight types (7%) of plants showed weak activity (±) of under 40% but over 20% inhibition ratio.

Therefore, one quarter of the tested plants suppressed the tumor promoter, okadaic acid. These samples showed that anti-OA activity might prevent the carcinogenesis. Overall, these findings show similar trends as in the reports of anti tumor-promoter activity against TPA, another type of tumor promoter (Koshimizu, 1995, 1996; Ohigashi, *et al.*, 1997), although the activity in each sample is different.

Physicochemical properties of the active component The physicochemical properties of the components of samples showing strong anti-OA activity were examined. For one week to determine stability of the active components in a refrigerator (4°C), as shown in Table 2, only burdock maintained the same level of activity (100%) as before. Basil still showed weak activity (38%), but the other plants lost activity even in cold storage.

The change in activity following heat treatment at 100°C for 5 min was also evaluated and, as shown in Table 3, edible burdock

Table 2. Change in anti-OA activity by low temperature storage.

	Fresh	1 week
Basil	++	±
Peppermint	++	–
Broccoli	++	–
Tomato	+	–
Edible burdock	++	++
Watermelon	++	–
Winter fungus	++	–
Oyster mushrooms	+	–
Cultivated mushrooms	+	–

Anti-OA activity, Very strong (++): over 80%, Strong (+): from 40% to 79%, Weak (±): from 20% to 39%, None (–): less than 19%.

Table 1. Anti-OA activities of edible plants.

Activity	Samples				
++	Basil	Peppermint	Carrot leaf	Edible burdock	Broccoli
	Nabana	Watermelon	Winter fungus		
+	Tomato	Cabbage	Ashitaba	Turnip	Cauliflower
	White gourd	Balsam pear	Pumpkin	Cherry	Pear
	Chestnut	Tree tomato	Oyster mushroom	Cultivated mushroom	Hen of the wood
	Agar-agar				
±	Komatsuna	Spinach	Pak-choi	Perilla	Eggplant
	Garden pea	Pomegranate	Kiwi fruits		
–	Celery	Chicory	Chinese cabbage	Chinese leek	Japanese butterbur
	Japanese horwort	Radish leaf	Nanjaku-hasai	Red cabbage	Kaiware radish
	Sunny letucce	Red malabar nightshade	Water-cress	Water dropwort	Turnip leaf
	Vitamin-na	Himawari-na	Chinese mustard	Garland chrysanthemum	Shantung green
	Asparagus	Bracken	Leek	Flower stalk	Garlic
	Onion	Petty onion	Red onion	Welsh onion	Royal fern
	Bamboo shoot	Bamboo shoot (menma)	Radish	Radish (hatsuka)	Wasabi
	Carrot	Ginger	Myoga	Lotus	Tomato
	Cucumber	Sweet pepper	Paprika	Summer squash	Tomapea
	Potato	Sweet potato	Taro	Taro (kyo-imo)	Taro (oya-imo)
	Bean sprouts	Kidney bean	Soy bean	Melon	Orange
	Unshiu orange	Navel	Litchi	Lime	Pummelo
	Sudachi	Yuzu	Lemon	Kabosu	Summer orange
	Grapefruit	Apple	European pear	Peach	Prune
	Grape (kyohou)	Strawberry	Blueberry	Akebia	Pineapple
	Banana	Matsutake	Nameko	Shiitake	Shimeji
	Hiziki	Kombu	Woody ear	Mekabu	Wakame

Anti-OA activity, Very strong (++): over 80%, Strong (+): from 40% to 79%, Weak (±): from 20% to 39%, None (–): less than 19%.

Table 3. Change in anti-OA activity by heat treatment.

	Fresh	Boiled
Basil	++	—
Peppermint	++	—
Broccoli	++	—
Tomyo	+	—
Cabbage	+	+
Watermelon	++	—
Edible burdock	++	++
Winter fungus	++	±
Oyster mushrooms	+	—
Cultivated mushrooms	+	—

Anti-OA activity, Very strong (++): over 80%, Strong (+): from 40% to 79%, Weak (±): from 20% to 39%, None (—): less than 19%.

Table 4. Anti-OA activities of the fractions from freeze dried samples that showed strong activity.

	Hexane	Benzene	Ethyl acetate	Methanol	Water
Broccoli	++	—	—	—	±
Edible burdock	++	+	—	—	++
Cabbage	++	++	+	+	++
Cultivated mushrooms	—	—	—	—	—
Pumpkin	—	—	±	—	+
Eggplant	—	—	±	—	++

Anti-OA activity, Very strong (++): over 80%, Strong (+): from 40% to 79%, Weak (±): from 20% to 39%, None (—): less than 19%.

(100%) and cabbage (55%) showed activity following this treatment, and winter fungus (37%) showed weak activity, but the other plants showed none.

These findings indicate that only edible burdock shows component stability through the processes of cold storage and boiling. Winter fungus, cabbage and basil maintained component stability in either process.

The solubility of the active components To fractionate the active components, 6 freeze dried samples that showed activity were extracted with 10 volumes of several different solvents for 20 min, and then each resulting extract was evaporated to dryness. The solvents used were hexane, benzene, ethyl acetate and methanol in order of polarity. After extraction with the first solvent three times, the residue was successively extracted with the next solvent. After each resulting extract was dissolved at a suitable concentration in DMSO, it was suspended in the buffer solution, and subjected to assay of anti-OA activity.

In the water-soluble fraction, edible burdock (83%), eggplant (80%), cabbage (85%) and pumpkin (63%) showed strong activity, as shown in Table 4, while cabbage, edible burdock and broccoli also showed strong activity in low polarity fractions like hexane. Notably, cultivated mushrooms showed no activity in any fraction, nor was activity seen in any mixture of them.

Furthermore, the activity in each solvent fraction following heating of cabbage and edible burdock, which showed activity after the heat treatment was examined. Although differences were apparent, the activity was present in each solvent fraction (Table 5). Representative water-soluble components such as ascorbic acid, thiamin and cystein that were contained in strong activity samples were tested (Science & Technology Agency, 1998). The anti-OA activity of ascorbic acid (1 mg/ml) was 15%, cystein (1 mg/ml) was 27% and thiamin (1 mg/ml) was 18%. All

Table 5. Anti-OA activities of the fractions from boiled samples that showed strong activity.

	Cabbage		Edible burdock	
	Fresh	Boiled	Fresh	Boiled
Hexane	++	+	++	+
Benzene	++	+	+	++
Ethyl acetate	+	±	—	—
Methanol	+	+	—	—
Water	++	±	++	++

Anti-OA activity, Very strong (++): over 80% Strong (+): from 40% to 79% Weak (±): from 20% to 39% None (—): less than 19%.

these water-soluble standards showed weak activity. The dietary fiber in these foods was also tested. For these edible plants which showed the activity, the active components may exist except for these standard substances. In the future, we will use purification techniques to clarify the chemical structure of the active components.

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