α-Amylase Inhibitors from Roselle (*Hibiscus sabdariffa* Linn.) Tea

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A roselle (*Hibiscus sabdariffa* Linn.) tea extract was found to have high inhibitory activity against porcine pancreatic α-amylase. Hibiscus acid and its 6-methyl ester were respectively isolated as active principles from the 50% methanol and acetone extracts of roselle tea. The activity of each isolate was compared to that of structurally related citric acid, a previously known inhibitor of fungal α-amylase.

**Key words:** porcine pancreatic α-amylase inhibitor; roselle; *Hibiscus sabdariffa*

According to the broadly accepted view, suppressing the activity of α-glucosidases, which are digestive enzymes in the intestinal lumen and in the brush border membrane, results in decreased glucose absorption and consequently reduces the postprandial blood glucose level.11 α-Glucosidase inhibitors from various sources have recently been isolated,2–10 and a growing number of them are being investigated not only in both animal tests and in human clinical trials.5–7 On the other hand, a few α-glucosidase inhibitors have been found to inhibit the replication of HIV due to an altered viral glycoprotein formation and decreased recognition of infected cells.8–10 These reasons have resulted in an extensive search for α-glucosidase inhibitors being carried out worldwide. Our research also attempts to isolate a porcine pancreatic α-amylase (PPA) inhibitor from Japanese and Thai edible plant materials. This present work studied roselle (*Hibiscus sabdariffa* Linn.) tea, which is made from dried flowers. Roselle juice is a popular beverage and a herbal medicine in Thailand and is claimed to be a thirst quencher, diuretic, gall stone disperser, antipyretic and cough reliever. The bioactive compounds from roselle extracts have been found to be effective in inhibiting mouse skin tumors11 and the angiotensin I-converting enzyme (ACE) *in vitro*,12 and some other extracted substances possessed antioxidative activity.13 However, no report has previously mentioned roselle in relation to α-glucosidases.

Aqueous methanol extracts of 43 foodstuffs from Thai and Japanese sources were screened for PPA inhabitation. The starch azure (2 mg, Sigma Chemical Co.), which was used as a substrate, was suspended in 0.2 ml of a 0.05 M Tris-HCl buffer (pH 6.9) containing 0.01 M CaCl₂ and boiled for 5 min. The starch solution was then pre-incubated at 37°C for 5 min. A test sample in 50% dimethyl sulfoxide (0.2 ml), and 0.1 ml of a PPA solution (Sigma Chemical Co.) in the above-mentioned buffer (2.11 unit/ml) were applied for each assay. The reaction was carried out at 37°C for 10 min and stopped by adding 0.5 ml of 50% acetic acid. The reaction mixture was then centrifuged at 3000 rpm for 5 min at 4°C. The absorbance of the resulting supernatant at 595 nm was measured. The α-amylase inhibitory activity was calculated as follows:

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\text{PPA inhibitory activity (\%)} = \frac{[A_{e+} - A_{c+} - (A_{t} - A_{b})]}{(A_{e+} - A_{c+})} \times 100,
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where \( A_{e+} \), \( A_{c+} \), \( A_{t} \), and \( A_{b} \) are defined as the absorbance of 100% enzyme activity (only the solvent with the enzyme), 0% enzyme activity (only the solvent without the enzyme), a test sample (with the enzyme), and a blank (a test sample without the enzyme), respectively.

Each plant material was extracted with 50% aqueous methanol (10 ml/g fr. wt.) for 24 h at room temperature. The methanol was evaporated from one part of the extract, the resulting residue being redissolved in dimethyl sulfoxide (10 ml/g fr. wt.) and subjected to a PPA inhibitory activity assay.

The screening results were as follows (% of inhibition): banana tea (35), benibana tea (2), ginnema tea (8), persimmon tea (38), balsam pear tea (1), kaffir lime (5), tarragon (13), juniper berries (0), green pepper (1), lotus root (0), ois root (12), maruba flower (8), alfalfa (8), rensoeno (3), eida (11), tara tree (4), sanzashi (17), neem tree (8), *Coccinia grandis* L. Voigt (14), lead tree (88), onion (0), nitta tree (0), lemon grass (0), pepper vine leaves (0), pepper vine stem (17), lesser eggplant (0), hairy basil (14), golden apple (27), roselle tea (100), ginger tea (11), pride of india leaves (51), pride of india seed (22), galangal leaves (1), guava leaves (60), tropical almond leaves (100), lotus (92), fragrant screwpine root (2) fragrant.

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screwpine medicine powder (0), boraped stem (0), boraped medicine powder (27), *Ganoderma lucidum* Fr. n Karst (6), *Tectona grandis* Linn. leaves (2), and *Orthosiphon grandiflorus* Bolding. The roselle tea (*Hibiscus sabdariffa* Linn.) extract showed in high inhibition against PPA and was chosen for isolation and purification of the active principles.

Ten gram of commercially available roselle tea (made from dried flowers and purchased from a local market in Bangkok, Thailand) was extracted with 50% methanol (10 ml/g dry wt.). The extract was partitioned between ethyl acetate and water. The active aqueous fraction was chromatographed in an ODS column (Cosmosil 75C18-OPN, Nacalai Tesque, 40 × 150 mm) with water-methanol gradient elution. The active fraction, which was eluted with 95:5 water-methanol, was purified by HPLC [column: Inertsil PREP ODS, 6 × 250 mm; mobile phase: water-methanol (95:9) containing 0.1% trifluoroacetic acid (v/v); flow rate: 1 ml/min; detection: UV 235 nm] to yield an active principle (I, 48 mg, tR = 17.5 min). The physicochemical properties of I were as follows. FD-MS m/z (%): 205 ([M + H]+, 100), 409 ([2M + H]+, 55); FD-HR-MS m/z: 205.0351 (calcd. for C12H16O2; 205.0348); 1H-NMR δ (D2O) ppm: 2.87 (1H, d, J = 18 Hz, 4α-H), 3.44 (1H, d, J = 18 Hz, 4β-H), 3.89 (3H, s, 6-Me), 5.44 (1H, s, 2-H); 13C-NMR δ (D2O) ppm: 44.9 (t, 4-C), 56.7 (q, 6-Me), 81.1 (s, 3-C), 86.6 (d, 2-C), 172.3 (s, 1-C), 174.5 (s, 6-C), 178.3 (s, 5-C). The purified active principle was identified as hibiscus acid 6-methyl ester (I) by spectroscopic evidence (Fig. 1). Its IC50 value for PPA was 3.22 mM.

In order to clarify whether I was derived from the plant material itself or obtained by esterification during the extraction step, the extraction method was modified. An acetone (100 ml) extract of roselle tea (10 g) was directly investigated by HPLC [column: Inertsil PREP ODS, 200 × 250 mm; mobile phase: water-methanol (95:5) containing 0.1% trifluoroacetic acid (v/v); flow rate: 7 ml/min; detection: UV 235 nm], resulting in an active compound (2, 60 mg, tR = 11.5 min), which had the following spectral characteristics. FD-MS m/z (%): 191 ([M + H]+, 100), 381 ([2M + H]+, 25); 1H-NMR δ (acetone-d6) ppm: 2.77 (1H, d, J = 17 Hz, 4α-H), 3.24 (1H, d, J = 17 Hz, 4β-H), 5.34 (1H, s, 2-H). The purified active substance was proven to be hibiscus acid (2). The IC50 value for the PPA inhibition of 2 was 1.10 mM. This result indicates that the actual PPA inhibitor was 2 due to its high content in the acetone extract and that 1 was probably derived from 2 by methylation during the first extraction procedure.

Hibiscus acid, a lactone form of (+)-allo-hydroxycitric acid, has been found in the calyxes of *Hibiscus sabdariffa* Linn. as the major constituent. The other naturally occurring isomer of hydroxycitric acid, (−)-hydroxycitric acid, has been isolated from *Garcinia cambogia*. This latter isomer is known as an inhibitor of ATP-citrate lyase resulting in the depression of fatty acid synthesis. The use of (−)-hydroxycitric acid in new therapy for obesity has now become possible. However, the biological activities of neither the former isomer nor its derived lactone form (hibiscus acid) have previously been studied. Our finding is the first report of hibiscus acid and its 6-methyl ester isolated from roselle tea possessing PPA inhibitory activity.

Consideration of the structure of compounds 1 and 2 led us to compare their inhibitory activity to that of citric acid which has been reported to inhibit fungal α-amylase. Citric acid at various concentrations was used to compare its PPA inhibitory activity to that of active principles 1 and 2 (Fig. 2). The IC50 value for citric acid toward PPA (0.91 mM) was lower than that of hibiscus acid (1.10 mM) and its 6-methyl ester (3.22 mM), and much lower than the value against fungal α-amylase.

We also measured the pH value of the reaction mixture for each PPA assay since the acidic nature of these acid solutions might simply have inhibited the enzyme activity. The IC50 concentration of citric acid, hibiscus acid, and hibiscus acid 6-methyl ester made the pH drop to 5.0, 4.9, and 4.8 in the respective reaction mixtures. The test for the pH sensitivity of PPA revealed that the activity of this enzyme was not significantly affected in a pH range from 3.5 to
7.0, but was severely inhibited at pH 3.0. This means that the inhibitory effect of these organic acids on PPA could not be attributed to the pH drop caused by their acidic properties.

Although it can be hypothesized that α-glucosidase inhibitors reduce glucose absorption, the possible effect of hibiscus acid (I) and its 6-methyl ester (2) on human the organism is not yet known. In order to study this, the inhibitory effect of each of these compounds on α-glucosidase enzymes by using a human colon carcinoma cell line (Caco-2) is being investigated.

References


