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Screening for α-amylase Inhibitory Activities of Woody Plants[*1]

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ABSTRACT

Inhibitors of α-amylase are important for the treatment of diabetes and obesity. Using enzyme inhibitor's activity, ethanolic extracts of 87 species in 12 families were screened and compared their inhibitory effect on α-amylase. As a results, we can find that extracts of *Distylium racemosum*, *Acer tegmentosum*, *Corylopsis veitchiana*, *Cornus walteri* and *Corylopsis spicata* showed higher α-amylase inhibitory activities than the others and have potential possibility of using control agents for carbohydrate-dependent disease.

Keywords : α-amylase inhibitors, woody plant, *Distylium racemosum, Acer tegmentosum, Corylopsis veitchiana, Cornus walteri, Corylopsis spicata*

1. INTRODUCTION

Carbohydrates are the largest components of the human diet. However, only monosaccharides can be absorbed by the small intestine. Polysaccharides and disaccharides, such as starch and sucrose, undergo rapid enzymatic degradation to monosaccharides through the action of pancreatic α-amylase and -glucosidases. As a consequence, a rapid rise in blood glucose level is generally observed after carbohydrate ingestion. Inhibition of α-amylase activity may be one way of treating diabetes and obesity (Svensson *et al.*, 2004).

α-Amylase (α→1-4-D-glucano-4-glucan hydrolase, E.C 3.2.1.1) is an endo-acting enzyme produced by animals, fungi, bacteria and plants (Yoon and Robyt 2003). There are two kinds of α-amylases produced by mammals. The first one is salivary α-amylase produced from the parotid gland. The second enzyme is pancreatic α-amylase is from the pancreas (Gyemant *et al.*, 2003).

The function of α-amylase inhibitor is to retard absorbtion of glucose by the inhibition of carbohydrate-hydrolysing enzyme in the digestive organs. This α-amylase inhibitor has a chemical structure similar to that of oligosaccharides derived from the digestion of starch. The inhibition of α-amylase results in delaying the digestion of carbohydrates, thereby reducing the rate of absorption of simple sugars and reducing postprandial blood glucose levels (Robert and Krol, 1997). The ability to digest car-

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bohydrate is useful in the treatment of non-insulin-dependent diabetes mellitus and obesity (Murai *et al.*, 2002).

There are reports on several established α-amylase inhibitors such as acarbose and voglibose from microorganisms, and nojirimycin and 1-deoxynojirimycin from plants. Furthermore, their effect on blood glucose levels after food uptake has been well documented. Two different α-amylase inhibitor families, naturally occurring proteins and substrates analogues, particularly pseudooligosaccharides have drawn much attention (Abell *et al.*, 1998). Chrzaszcz and Janicki (1933) first reported such as α-amylase inhibitor from buckwheat. Many studies have also been focused on screening such α-amylase inhibitor from plants including barley, rye, bean, legumes and oriental drugs (Moon *et al.*, 1995; Chun *et al.*, 2001). Yamada *et al.* (2001) isolated two α-amylase inhibitors from seeds of tepary bean. However, no such attempts to screen α-amylase inhibitor from woody plants have been made. Unlike crop and herbal plants, woody plants are typically perennials and exposed to varying temperature. Therefore they contain diverse compounds including secondary metabolites, cell wall compounds and defensive materials.

We therefore measured the inhibitory effect of ethanol extracts from more than 2,000 species of woody plants on α-amylase. In the present report, we compared the inhibitory effect of ethanol extracts from only 87 species of woody plants on α-amylase in other to search for α-amylase inhibitors.

2. MATERIALS and METHODS

2.1. Materials

Eighty-seven species in 12 families were collected from Chollipo arboretum, Taean, Chungnam and Korea Forest Research Institute, Suwon, Korea.

2.2. Chemicals

α-Amylase was purchased from Sigma (Mo., USA). Soluble starch was obtained from Yakuri Pure Chemicals CO.

2.3. Preparation of Extracts

Whole plants were dried and ground to fine powder by Wiley mill. Wood powder (10 g) was then extracted with 70% ethanol (100 mℓ). The evaporated extracts were used as crude samples.

2.4. α-amylase Inhibitory Activity Test

Salivary α-amylase (50 μℓ, 1.0 units), extracts solution (50 μℓ, 500 ppm) and 0.2 M Tris-HCl buffer (pH 7.0, 500 μℓ) were preincubated at 27°C for 5 min and then 0.5% starch (250 μℓ) was added. After 10 min incubation at 27°C, the reaction was stopped by adding 25% trichloroacetic acid (TCA). After mixing with iodine, the optical density was measured at 625 nm (Helrch, 1990). As a control, 70% ethanol was used. The inhibitory activity was calculated as follow;

α-amylase inhibitory acticity (%) = [(OD_{625} of control - OD_{625} of sample)/ OD_{625} of control] $\times 100$

and expressed the results were as follow:
-: no inhibition; +: 10～20% inhibition; ++: 20～50% inhibition; +++: 50～80% inhibition; ++++: 80～100% inhibition.

3. RESULTS and DISCUSSION

The inhibitory activities of α-amylase by the extracts from 87 species were examined and shown in Tables 1, 2, and 3.

Table 1. α-Amylase inhibitory activities of 6 families of woody plants

Family	Species	Inhibition
Aceraceae	*Acer cappadocicum* var. sinicum	+++
	Acer diabolicum	++++
	Acer distylum	++++
	Acer insularis	++++
	Acer okamotoanum	++++
	Acer tegmentosum	++++
Caprifoliaceae	*Lonicera japonica* cv. Aureoreticulata	++++
	Viburnum plicatum cv. Lanarth	++++
	Viburnum acerifolium	++++
	Viburnum awabukki	+++
	Viburnum dilatatum	+++
	Viburnum erosum var. taguetii	++++
Cornaceae	*Cornus florida*	++++
	Cornus popliophylla	++++
	Cornus purpusii	++++
	Cornus rugosa	+++
	Cornus sanguinea	+++
	Cornus walteri	++++
Salicaceae	*Salix alba* cv. Yellow Stem	++++
	Salix chinensis	++++
	Salix purpurea var. smithiana	++++
	Salix sachalinensis	++++
	Salix sinensis	++++
Ericaceae	*Arbutus unedo*	++++
	Arbutus unedo cv. Rubra	++++
	Erica lusticana	++++
	Erica x williamsii	++++
	Kalmia latifolia	++++
	Lyonia ovalifolia var. elliptica	++++
	Pieris formosana cv. Caerhays	++++
	Rhododendron atlanticum	+++
	Rhododendron indicum cv. Flame Creeper	++++
	Rhododendron indicum	+++
	Rhododendron japonicum	++++
	Rhododendron simsii	++++
	Rhododendron wilsonii	++++

Table 1. (continued)

Family	Species	Inhibition
Hamamelidaceae	*Corylopsis coreana*	++++
	Corylopsis gotoana	++++
	Corylopsis spicata	++++
	Corylopsis veitchiana	++++
	Corylopsis willmotiae cv. Spring Purple	++++
	Distylium racemosum	++++
	Hamamelis japonica var. obtusata	++++
	Hamamelis molis cv. Brevipetala	++++
	Hamamelis virginiana	++++
	Liquidambar styraciflus cv. Aurora	++++
	Loropetalum chinense	++++
	Parrotia persica	++++

-: no inhibition; +: 10~20% inhibition; ++: 20~50% inhibition; +++: 50~80% inhibition; ++++: 80~100% inhibition.

Table 2. α-Amylase inhibitory activities of 2 families of woody plants

Family	Species	Inhibition
Fabaceae	*Campylotropis macrocarpa*	+
	Cercis chinensis	++++
	Cercis chinensis cv. Alba	++++
	Cercis chinensis cv. Avondale	++++
	Cercis racemosa	++++
	Lespedeza bicolor	++++
	Pueraria thunbergiana	-
	Robinia pseudoacasia cv. Umbraculifera	-
	Robinia pseudoacasia cv. Tortuosa	-
	Sophora japonica	-
Taxodiaceae	*Cryptomeria japonica* cv. Pygmaea	-
	Cryptomeria japonica cv. Yoshion	+
	Cryptomeria japonica cv. Dacrydioides	++++
	Cryptomeria japonica cv. Globosa Nana	+
	Metasequoia glyptostroboides	-
	Sciadopitys verticillata	++
	Taxodium distichum	++
	Taxodium distichum cv. Penens	+++

-: no inhibition; +: 10~20% inhibition; ++: 20~50% inhibition; +++: 50~80% inhibition; ++++: 80~100% inhibition.

Table 3. Twenty one woody species and their family showing α-amylase inhibiting activities

Family	Species	Inhibition
Euphorbiaceae	*Daphniphyllum macropodum*	-
	Mallotus japonicus cv. Variegata	-
	Mallotus japonicus	-
	Sapium japonicum	-
	Sapium sebiferum	-
Lauraceae	*Cinnamomum japonicum*	++
	Lindera erythrocarpa	++
	Lindera obtusiloba	+
	Machilus thunbergii	++
Lythraceae	*Lagerstroemia indica*	+
	Lagerstroemia indica for. alba	-
	Lagerstroemia indica X *fauriei*	-
	Lagerstroemia limi	++
	Lagerstroemia subcostata	-
Magnoliaceae	*Kadsura japonica*	-
	Liriodendron tulipifera	-
	Magnolia campbellii subsp. Mollicomata	-
	Magnolia compressa	-
	Magnolia grandiflora	-
	Magnolia salicifolia	-
	Magnolia stellata cv. Rosea	-

-: no inhibition; +: 10~20% inhibition; ++: 20~50% inhibition; +++: 50~80% inhibition; ++++: 80~100% inhibition.

In Table 1, the plant species with the most effective α-amylase inhibit activity were described. The ethanolic extracts of plants belonging to 6 families, Aceraceae, Caprifoliaceae, Cornaceae, Salicaceae, Ericaceae and Hamamelidaceae showed high inhibitory activities (more than 50%) aganist α-amylase. Among them, *Distylium racemosum, Acer tegmentosum, Corylopsis veitchiana, Cornus walteri* and *Corylopsis spicata* had more than 95% inhibitory activities against α-amylase.

Table 2 showed the list of most effective or less effective species in 2 families; Fabaceae and Taxodiaceae. Among to trees belonging to the family Fabaceae, *Cercis chinensis, Cercis chinensis* cv. Alba, *Cercis chinensis* cv. Avondale, *Cercis racemosa* and *Lespedeza bicolor* showed high inhibitory activities (more than 80%), whereas *Pueraria thunbergiana, Robinia pseudoacasia* cv. Umbraculifera, *Robinia pseu-*

doacasia cv. Tortuosa and *Sophora japonica* have no inhibitory activities on α-amylase at all.

As shown in Table 3, the plants in the families of Euphorbiaceae, Lauraceae, Lythraceae and Magnoliaceae have no or low inhibitory activities (less than 50%) on α-amylase. In the case of those belonging to the families, Euphorbiaceae and Magnoliaceae, no inhibitory activities were detected.

Kim *et al.* (2004) compared pine bark extracts with acarbose and voglibose for their inhibitory activity against α-amylase. They concluded that *Pinus densiflora* bark extracts showed the highest inhibition activity against several carbohydrates-hydrolysing enzymes. Lee and Yang (1988) examined several oriental drugs to find amylase inhibitors and found that *Areca catechu* L., *Cinnamomum cassia* and *Ephedra sinica* Strapf had stronger inhibitory activities against α-amylase than the other oriental drugs. In their studies, few oriental drugs of animal and mineral origins had the inhibitory activity against α-amylase. According to Iwuoha and Aina (1997), the presence of phenolics may reduce amylolytic activity. They also reported that phenolics have the capacity to inhibit amylase activity. Therefore the decrease in tannin content may reduce α-amylase inhibitors (Abd El-Haddy and Habiba, 2003).

The structural configuration of α-amylase inhibitor competitively allows to inhibit α-amylase. The inhibitors of α-amylases have applications in modifying and controlling α-amylase action. Because of this reaction, they may also be useful for the treatment of metabolic diseases.

Based on the α-amylase inhibition activities of some woody plants extracts found in the present study, we were able to screen several promising tree species that may be useful for carbohydrate-dependent disease. Those trees are the members of the families Aceraceae, Caprifoliaceae, Cornaceae, Salicaceae, Ericaceae and Hamamelidaceae.

4. CONCLUSIONS

In search for α-amylase inhibitors from natural products, we measured the α-amylase inhibitory activities of the extracts of 87 species of woody plants. As results, we can conclude that *Distylium racemosum, Acer tegmentosum, Corylopsis veitchiana, Cornus walteri* and *Corylopsis spicata* showed higher α-amylase inhibitory activities than others. It is also need to continue a series of study on the isolation and purification of inhibitory substance from woody plants.

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