J. Korean Wood Sci. Technol. 43(2): 238~247, 2015 pISSN: 1017-0715 eISSN: 2233-7180
http://dx.doi.org/DOI : 10.5658/WOOD.2015.43.2.238

α-Glucosidase Inhibition Activity of the Extracts of Katsura Tree (*Cercidiphyllum japonicum* Sieb. Et Zucc) Leaves[1]

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ABSTRACT

Katsura tree (*Cercidiphyllum japonicum* Sieb. Et Zucc) leaves were collected, air-dried and extracted with 70% aqueous acetone, then concentrated and sequentially fractionated using *n*-hexane, CH_2Cl_2, EtOAc, and H_2O to be freeze dried for antioxidant and α-glucosidase inhibition activity tests.

The antioxidant activity of the extracts was evaluated using DPPH (1,1-diphenyl 2-picrylhydrazyl) free radical scavenging assay. The test concentrations were adjusted to 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.9, 1.95 and 0.97 ppm.

The H_2O and EtOAc fractions showed higher activities compared with the control, α-tocopherol, at all concentrations. The crude fraction also gave better activity at the concentrations lower than 62.5 ppm. However, the nonpolar *n*-hexane and CH_2Cl_2 fractions gave prominently lower activities compared with the control at all concentrations.

The IC_{50} values of the crude, EtOAc, and H_2O fractions exhibited 11.78, 4.29 and 9.80 μg/mℓ, respectively, compared with 12.08 μg/mℓ of the control. But the *n*-hexane and CH_2Cl_2 fractions indicated 300 and 91.85 μg/mℓ of IC_{50}, respectively.

α-Glucosidase inhibition activity was evaluated at the concentrations of 50, 25, 12.5, 6.3, 3.1, 1.6 and 0.8 ppm.

The inhibition activities were increased according to as the increase of sample concentrations. However, the nonpolar *n*-hexane and CH_2Cl_2 fractions indicated very low inhibition activities compared with acarbose, a positive control. The EtOAc fraction showed very good capability as almost 100% compared with the control at the higher concentrations than 12.5 ppm and the crude fraction also indicated good potential as 95% and 100% at 25 and 50 ppm, respectively. The H_2O fraction gave good inhibition value as 90% at 50 ppm although the value was lower than the control. These results showed that the polar fractions had better α-glucosidase inhibition activities.

The IC_{50} values of the nonpolar fractions, *n*-hexane and CH_2Cl_2, showed very lower values as 468 and 103.6 μg/mℓ, respectively, than the control.

[1] Date Received January 27, 2015, Date Accepted February, 24, 2015
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However, the polar fractions, crude, EtOAc and H_2O, showed 7.1, 3.7 and 13 μg/mℓ, respectively, indicating that these fractions can be used as natural bioresources for treating diabetes mellitus.

Also α-glucosidase inhibition activity had a positive correlation with antioxidant activity of the extracts.

Keywords : Katsura tree (*Cercidiphyllum japonicum*) leaves, EtOAc fractionation, diabetes, α-glucosidase inhibition activity

1. INTRODUCTION

Katsura tree (*Cercidiphyllum japonicum* Sieb. Et Zucc), is the only species belonging to *Cercidiphyllum* genus, which is well represented in the fossil record, with occurrences in the late Cretaceous and Tertiary of North America and Europe. However, it is now confined to East Asian countries (Manchester *et al*. 2009). The tree is a long-lived, deciduous, wind-pollinated tree with dimorphic leaves and up to 30 to 45 m tall with a symmetrical canopy and new growth is reddish turning a light pale green. Fall color is a spectacular yellow, with some red. Thus, it is valued as an ornamental or a shade tree for landscape (Zhang *et al*. 2009). It is also a commercially and ecologically valuable one and likely to become one of the medicinal tree species. The clustered pod-like fruits contain numerous small seeds which adapted for wind dispersal. The natural populations of the tree inhabit distribute sites (600 to 2000 m) of temperate deciduous forests scattered across East China and Japan (Isagi *et al*. 2005). Because of its extremely low ability of regeneration in natural population, the number of its populations is very little. Therefore, the tree is now treated as “endangered” in China and recognized globally as lower risk under the International Union for the Conservation of Nature criteria.

Diabetes mellitus is a common disease with many complications such as atherosclerosis, cardiac dysfunction, retinopathy, neuropathy, and nephropathy (Sowers *et al*. 2001). α-Glucosidase (EC 3.2.1.20) catalyzes the final step in the digestive process of carbohydrates. Its inhibitors can retard the uptake of dietary carbohydrates and suppress postprandial hyperglycemia and could be useful for treating diabetic and/or obese patients (Toeller 1994). α-Glucosidase inhibitors such as acarbose, miglitol, and voglibose are known to reduce postprandial hyperglycemia primarily by interfering with the carbohydrate digestive enzymes and by delaying glucose absorption. Aldose reductase (E.C.1.1.1.21, AR) is the first enzyme in the polyol pathway; it catalyzes the reduction of D-glucose from the aldehyde form into D-sorbitol with concomitant conversion of NADPH to NADP+ (Kador *et al*. 1985a, b). It is generally accepted that this polyol pathway plays an important role in the development of some degenerative complications of diabetes. The elevated blood glucose level, a characteristic of diabetes mellitus, causes significant fluxes of glucose through the polyol pathway in tissues such as nerves, retina, lens, and kidneys, where glucose

uptake is independent of insulin (Chihiro 1998). Thus, AR inhibitors have attracted attentions in therapeutic researches of diabetic complications

The inhibitory effects of plant phytochemicals, including polyphenols, against carbohydrate hydrolyzing enzymes contribute to the lowering of postprandial hyperglycemia in diabetic management as observed in vivo (Griffiths and Moseley 1980). Further evidence that polyphenolic compounds is linked to prevention of diabetic complications stems from in vivo studies with diabetic rats; polyphenolic compounds in plant materials are capable of reducing oxidative stress by scavenging reactive oxygen species and preventing cell damage (Fukuda *et al*. 2004). Also the polyphenolic compounds in edible plants are currently regarded as natural antioxidants, and their antioxidant activities are important for human health (Sabu *et al*. 2002).

Plants constitute a rich source of bioactive chemicals (Kador *et al*. 1985a, 1985b; Williamson *et al*. 1992). Since many plants are largely free from adverse effects and have excellent pharmacological actions, they could possibly lead to the development of new classes of safer antidiabetic agents or diabetic complication resolving agents. In addition, some flavonoids and polyphenols as well as sugar derivatives are found to be effective in inhibiting α-glucosidase and aldose reductase (Haraguchi *et al*. 1996; Lee and Kim 2001). Therefore, much effort has been focused on plants to produce potentially useful products such as commercial α-glucosidase inhibitors and aldose reductase inhibitors or lead compounds.

Recently there have been many studies to evaluate biological activities of various natural resources, including plants and tree species, and to develop pharmaceutical or functional food or cosmetic products. However, there are little studies on katsura tree extracts for developing functional and pharmaceutical products.

This work was to evaluate antioxidant and antidiabetic activity of the extracts of katsura tree leaves for future functional and pharmaceutical use of the species.

2. MATERIALS and METHODS

2.1. Plant material

Fresh *Cercidiphyllum japonicum* leaves were collected at Hwacheon, Gangwon-do in August 2013, air dried for two weeks and then ground to fine particles to be extracted.

2.2. Extraction and fractionation

The ground leaves (3 kg) were immersed in 70% aqueous acetone at room temperature for 3 days. After three times extraction and filtration, the filtrates were combined together and evaporated on a rotary evaporator under the reduced pressure at 40℃. The aqueous crude residue was successively fractionated on a separatory funnel and freeze dried to give *n*-hexane (CJLH, 2.6 g), CH_2Cl_2 (CJLM, 8.82 g), EtOAc (CJLE, 35.22 g), and H_2O (CJLW, 45.18 g) soluble fractions.

2.3. DPPH free radical scavenging activity assay

The assay was conducted as described by Li and Seeram (2010) and Wan *et al*. (2012). 100 μℓ of aliquots of the samples at ten concentrations (500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.9, 1.95 and 0.97 ppm) and α-tocopherol (positive control) were put into a 96-well micro-plate and 100 μℓ of DPPH (0.2 m*M*) was added to each well. The mixture was then incubated in the dark for 30minutes. The absorbance was measured at 517 nm with a micro-plate reader (Sunrise, TECAN) after 30 min. reaction in the dark.

The scavenging capacity (SC) was calculated as SC% = [(Ao-As)/Ao] × 100 where Ao is the absorbance of the reagent blank and As is the absorbance of the test sample. All tests were repeated three times. The result was expressed as IC_{50} value, that denotes the concentration of sample required to scavenge 50% DPPH free radicals.

2.4. α-Glucosidase inhibition activity assay

2.4.1. Preparation of α-glucosidase

The small intestine from a hog was collected immediately after slaughter and stored for further use. The intestine was opened, rinsed with distilled water three times, and the mucosa was scrapped off with a glass microscope slide. The collected mucosa was homogenized with S-10 direct driven stirrer (Hana Instruments) for 5 min. after five times addition of the mixture of 0.5 M NaCl, 0.5 M KCl and 5 mM EDTA (pH 7.0) based on the weight of mucosa, and then centrifuged. The centrifugal precipitate was treated with 5 mM EDTA and centrifuged (× 20,000 g) three times again. The obtained precipitate was homogenized by five times addition of 0.9 % NaCl solution based on the weight of the precipitate, and centrifuged (× 2,000 g) for 30 min. again. Then the supernatant was dialyzed for 24 hr. Protein was determined by the method of Bradford (Schmidt *et al*. 1979).

2.4.2. Analysis of α-glucosidase activity

In order to test the activity, α-glucosidase was reacted in a incubator containing substrate (4 mM maltose, 500 μℓ), 400 μℓ H_2O and 100 μℓ enzyme solution at 37℃ for 60 min. Then the mixture was treated in water bath at 100℃ for 5 min. Glucose was measured using Glucose (GO) Assay Kit (Sigma GAGO20-1KT). The enzyme activity was calculated by 1 unit, that denotes the production of 1 *uM* glucose for 1 min.

2.4.3. Evaluation of α-glucosidase inhibition activity

Each fraction of the extracts was diluted with eight concentrations (0.8, 1.6, 3.2, 6.3, 12.5, 25, 50 and 100 ppm) in 70 % EtOH. 50 μℓ of each diluted sample and 100 uℓ of α-glucosidase (10.2 unit/mℓ) were incubated in advance together at 37℃ for 10 min. After addition of 500 μℓ of the substrate (4 mM maltose), the mixture was reacted at 37℃ for 60

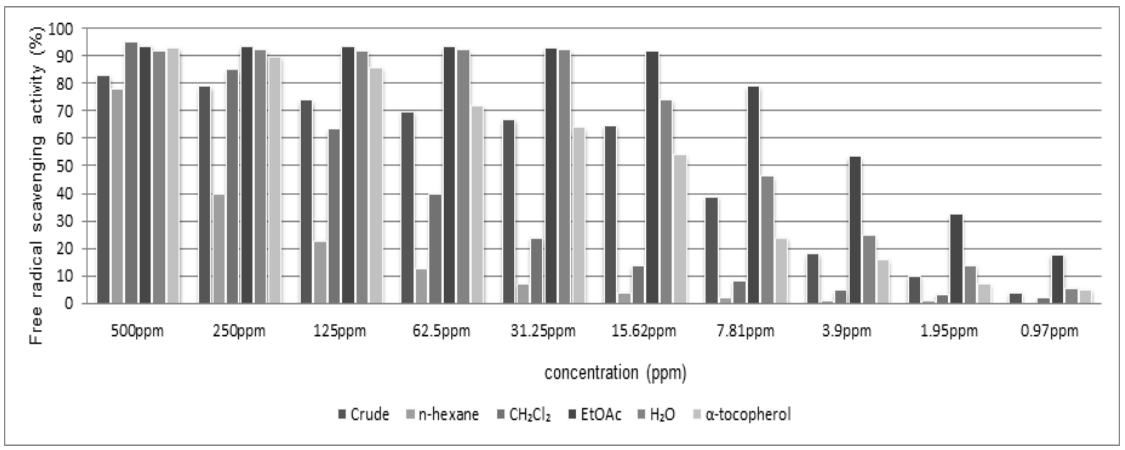

Fig. 1. DPPH free radical scavenging activities by the fractions of katsura tree leaves extracts.

Table 1. IC_{50} values of the antioxidant activities by the fractions of katsura tree leaves extracts

Samples		IC_{50} (μg/mℓ)
Fraction	Crude extract	11.78
	n-hexane fraction	300.00
	CH_2Cl_2 fraction	91.85
	EtOAc fraction	4.29
	H_2O fraction	9.80
Positive control	α-tocopherol	12.08

min. and then finished after the reaction at 100℃ for 5 min. Glucose was measured by Glucose (GO) Assay Kit and compared with the control, the solution reacted in 70% EtOH. The α-glucosidase inhibition activity of the test fraction was expressed as percentage (%) of inhibition and calculated as % inhibition of sample = $[(a_n-a_s)/a_n] \times 100\%$ where a_n is the difference in absorbance of the negative control and all the blanks while a_s is the difference in absorbance of the sample and all the blanks.

3. RESULTS and DISCUSSION

3.1. Free radical scavenging activity of the extracts

Antioxidant is able to stabilize or deactivate the free radical which cause oxidative damage to cellular structures. Free radical scavenging is performed by donating hydrogen to the free radical. Thus, free radical scavenging activity can be used to determine the antioxidant capacity.

DPPH free radical scavenging activity of the extracts of katsura tree (*Cercidiphyllum japonicum*) leaves was evaluated to determine the antioxidant capacity. The activity was expressed as IC_{50} value that means the concentration of antioxidant required to scavenge 50% of the DPPH radical.

The DPPH free radical scavenging activities and IC_{50} values for the five fractions of the extracts are shown in Fig. 1 and Table 1.

The activities were in proportion to the increase of the sample concentrations. In Fig. 1, the H_2O and EtOAc fractions of the extracts indicated higher activities compared with control value at all concentrations. The crude fraction also showed good activity compared with the control at the concentrations lower than 62.5 ppm. However, the nonpolar *n*-hexane and CH_2Cl_2 fractions gave prominently lower activities compared with the control at all concentrations.

In Table 1, the lower IC_{50} value implies the higher free radical scavenging capability. The crude, EtOAc, and H_2O fractions exhibited 11.78, 4.29 and 9.80 μg/mℓ of IC_{50} values, respectively, compared with 12.08 μg/mℓ of the control. But the *n*-hexane and CH_2Cl_2 fractions indicated high values as 300 and 91.85 μg/mℓ, respectively.

These results also coincide with the facts that polyhydroxy aromatic compounds are able to reduce DPPH through their hydrogen-donating ability (Mandal *et al*. 2009), and polyphenolic compounds is linked to prevention of diabetic complications stems from *in vivo* studies with diabetic rats; polyphenolic compounds in plant materials are capable of reducing oxidative stress by scavenging reactive oxygen species and preventing cell damage (Fukuda *et al*. 2004). Also the polyphenolic compounds in edible plants are currently regarded as natural antioxidants and their antioxidant activities are important for human health (Sabu *et al*. 2002). The less polar EtOAc fraction also contains the less polar phenolic aglycones such as isoflavones, flavanones, or flavones and flavonols (Herz *et al.* 1972, Thomas and Mabry 1968) and this fact may have a correlation with the good antioxidant activity of the EtOAc fraction of katsura tree leaves.

According to these results, the extracts of katsura tree leaves can be used as a natural resource having a big potential for natural antioxidant.

3.2. α-Glucosidase inhibition activity of the extracts

α-Glucosidase is one of the key enzymes involved in dietary carbohydrate digestion in human. It hydrolyzes the carbohydrate, releasing glucose and causes the raised postprandial blood glucose level. Inhibition to this enzyme can effectively descent the postprandial blood glucose level. This is especially beneficial for diabetic patients.

There are many synthetic drugs available to prevent or to treat diabetes such as acarbose, vogibose and miglitol. However, they usually can cause hepatic disorders and other negative gastrointestinal symptoms. Therefore, α-glucosidase inhibitors from natural source have become more preferable as means to prevent or to treat diabetes.

In this study, α-glucosidase inhibition activity of the extracts of katsura tree leaves was evaluated and the results were shown in Fig. 2 and Table 2.

In Fig. 2, the inhibition activities were increased according as the increase of sample

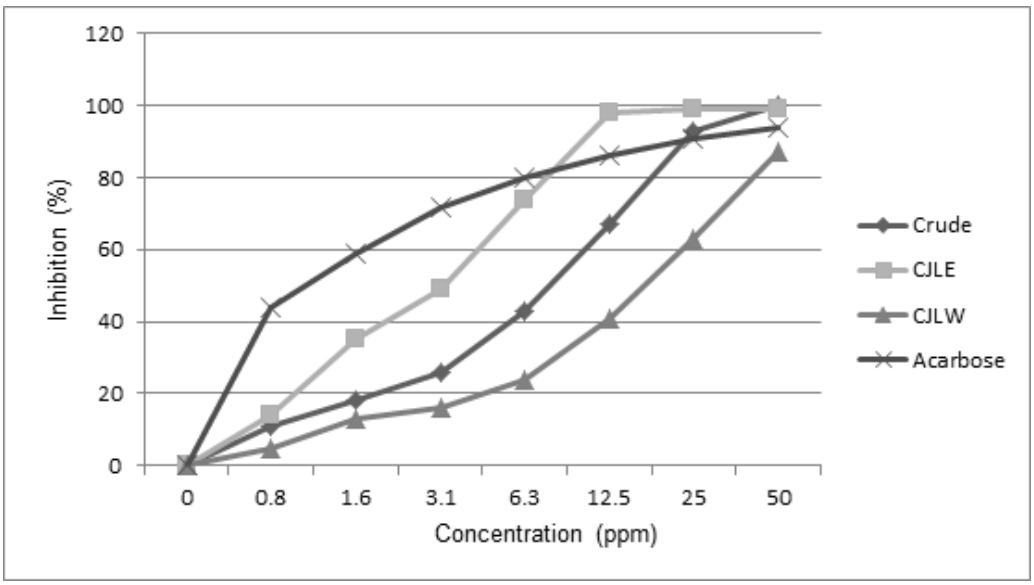

Fig. 2. α-Glucosidase inhibition activities by the fractions of katsura tree leaves extracts. α-Glucosidase inhibition activities by the fractions of katsura tree leaves extracts.

Table 2. IC_{50} values of α-glucosidase inhibition activities by the fractions of katsura tree leaves extracts

Samples		IC_{50} (μg/mℓ)
Fraction	Crude extract	7.1
	n-hexane fraction	468.0
	CH_2Cl_2 fraction	103.6
	EtOAc fraction	3.7
	H_2O fraction	13.0
Positive control	Acarbose	1.7

concentrations. However, the nonpolar *n*-hexane and CH_2Cl_2 fractions indicated very low inhibition compared with acarbose, the positive control. The EtOAc fraction showed very good capability as almost 100% compared with the control at the higher concentrations than 12.5 ppm and the crude fraction also indicated good potential as 95% and 100%, at 25 and 50 ppm, respectively, compared with the control at 25 and 50 ppm. The H_2O fraction too gave good inhibition value as 90% at 50 ppm although the value was lower than the control.

These results suggest that the polar fractions containing a large amount of polyphenolic compounds indicates a similar good α-glucosidase inhibition activitiy to the results of the antioxidant activity of the extracts of katsura tree leaves (Fukuda *et al*. 2004; Sabu *et al*. 2002; Mandal *et al*. 2009).

The IC_{50} values in Table 2 also indicated the similar values to the results of Fig. 2. The nonpolar *n*-hexane and CH_2Cl_2 fractions showed very lower values as 468 and 103.6 μg/mℓ than the positive control, but the polar crude, EtOAc and H_2O fractions gave 7.1, 3.7 and 13 μg/mℓ of IC_{50} values, respectively, indicating that

these fractions had a good potential as a natural resource for treating diabetes mellitus.

4. CONCLUSION

Katsura tree (*Cercidiphyllum japonicum* Sieb. Et Zucc) leaves were collected, air-dried and extracted with 70% aqueous acetone. The extracts were concentrated and then sequentially fractionated using n-hexane, CH_2Cl_2, EtOAc, and H_2O to be freeze dried for antioxidant and α-Glucosidase inhibition activity tests.

The antioxidant activity was evaluated using DPPH free radical scavenging assay. The concentrations of the samples and α-tocopherol, a positive control were adjusted to the concentrations of 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.9, 1.95 and 0.97 ppm.

The H_2O and EtOAc fractions showed higher activities compared with the control at all concentrations. The crude fraction also showed good activity at the lower concentrations than 62.5 ppm. However, the nonpolar *n*-hexane and CH_2Cl_2 fractions gave prominently lower activities compared with the control at all concentrations.

The IC_{50} values of the crude, EtOAc, and H_2O fractions exhibited 11.78, 4.29 and 9.80 μg/mℓ, respectively, compared with 12.08 μg/mℓ of the control. But the *n*-hexane and CH_2Cl_2 fractions indicated 300 and 91.85 μg/mℓ of IC_{50}, respectively.

α-Glucosidase inhibition activity of the extracts was evaluated at the concentrations of 50, 25, 12.5, 6.3, 3.1, 1.6 and 0.8 ppm.

The inhibition activities were increased according as the increase of sample concentrations. However, the nonpolar *n*-hexane and CH_2Cl_2 fractions indicated very low inhibition activities compared with acarbose, the positive control. The EtOAc fraction showed very good capability as almost 100% compared with the control at higher concentrations than 12.5 ppm and the crude fraction also indicated good potential as 95% and 100% at 25 and 50 ppm, respectively, compared with the control. The H_2O fraction gave good inhibition value as 90% at 50 ppm although the value was lower than the control.

These results indicate that the polar fractions containing a large amount of polyphenolic compounds have better α-glucosidase inhibition activities and that the antioxidant and antidiabetic activities have a positive correlation.

The IC_{50} values of the nonpolar fractions, *n*-hexane and CH_2Cl_2, were very low as 468 and 103.6 μg/mℓ than acarbose.

However, the polar fractions, crude, EtOAc and H_2O, gave 7.1, 3.7 and 13 μg/mℓ of IC_{50} values, respectively, indicating that these fractions can be used as a natural resource for treating diabetes mellitus.

According to the above results, the extracts of katsura tree leaves can be used as a potential natural resource substituting with synthetic antioxidant or antidiabetic medicine. Also there should be further studies to investigate the index compounds concerning in the biological activities and to make functional or medicinal output using the species.

ACKNOWLEDGEMENTS

This study was supported by the Basic Research Program for Forest Science of Korean Forest Service (No S211314L010130) and also partially by 2014 Research Grant from Kangwon National University (No 120141454).

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