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Original Research Article

Carbohydrate, Lipid Inhibitory Activity and Antioxidant Activity of Extracts from Several Economic Resource Plants *in Vitro*

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Abstract - The objective of this study was determined to evaluate α-amylase, α-glucosidase, pancreatic lipase inhibition *in vitro* and DPPH radical scavenging activity of the several Korean resources plants. The α-amylase inhibitory activity of *Salicornia herbacea*, *Erythronium japonicum* (flower) and *Phragmites communis* (root) in water extract showed relatively high 62.8%, 66.5% and 69.3%, respectively. The α-amylase inhibitory activity of *Citrus junos* (pericarp) and *Cornus officinalis* in methanol extract was found to have an effect with 32.8% in *Citrus junos* (pericarp) and 60.9% in *Cornus officinalis. Corylopsis coreana* in both water and methanol extract had the highest α-glucosidase inhibitory activity of 81.7% and 89.5%, while the extract of *Portulaca oleracea*, *Ficus carica* and *Citrus junos* was not measured α-glucosidase inhibitory activity at given experiment concentration. Depending on the extraction solvent and the plant species, it was observed that there was a significant difference in α-glucosidase inhibitory activity. The pancreatic lipase inhibitory activity showed relatively higher in the methanol extract than water extract except pericarp of *Citrus junos*. The DPPH radical scavenging activity of selected plants was much difference between measured plant species, and showed that the increase was proportional to the concentration. These results suggested that selected plants had the potent biological activity on carbohydrate, lipid Inhibitory activity and antioxidant activity, therefore these plant resources could be a good materials to develop medicinal preparations, nutraceuticals or health functional foods for diabetes or obesity.

Key words - α-Amylase, α-Glucosidase, Pancreatic lipase, Enzyme inhibition, DPPH radical scavenging activity

Introduction

Many traditional medicinal plants for diabetes and hyperlipidaemia have been reported in Korea, but the studies on anti-diabetic effects of these plants were not focused. Screening of α-amylase and α-glucosidase inhibitors from medicinal plants has received much attention throughout the world. This study was designed study the *in vitro* anti-diabetic and anti-hyperlipidaemia activity of 11 kinds of resource plants extract and to understand how the extract act against α-amylase, α-glucosidase and lipase activity. α-Amylase inhibitors are also known as starch blockers because they contain substances that prevent dietary starches from being absorbed by the body. Thus this could be useful in the treatment of obesity and diabetes mellitus. They exert their blood glucose lowering effect through the inhibition of an enzyme such as salivary and pancreatic amylase (Frantz *et al.,* 2005). Starches are complex carbohydrates that cannot be absorbed unless they are first broken down by the digestive enzyme amylase and other secondary enzymes (Marshall *et al.,* 1975). In other words, α-amylase and α-glucosidase are enzymes involved in starch breakdown and intestinal absorption, respectively, that is, α-amylase is involved in the digestion of carbohydrates to produce simpler saccharides, whereas the α-glucosidase is involved in their absorption. Inhibition of these two enzymes would result in a lower blood

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glucose levels after a rich carbohydrate diet. Acting as a key enzyme for carbohydrate digestion is intestinal α-glucosidase, a glucosidase secreted in the epithelium of the small intestine. α-Glucosidase has been recognized as a therapeutic target for the modulation of postprandial hyperglycemia, which is the earliest metabolic abnormality that occurs in NIDDM (Non-insulin dependent diabetes mellitus) (Kim *et al.,* 2005). α-Glucosidase inhibitors such as acarbose, miglitol, and voglibose are known to reduce postprandial hyperglycemia primarily by interfering with the activity of carbohydrate- digesting enzymes and delaying glucose absorption. In addition, numerous α-glucosidase inhibitors have been extracted from plants, which are of clinical importance (Yoshikawa *et al.,* 1998; Nishioka *et al.,* 1998). Specific enzyme inhibitors are biochemical tools that have potential utility in the treatment of diseases. α-amylase and α-glucosidase inhibitors are drug targets for the treatment of diabetes, obesity and hyperlipidaemia. Obesity is a known health condition and the prevalence is rising globally (Mokdad *et al.,* 1999; Yoshiike *et al.,* 1998; Ogden *et al.,* 2006). Weight loss and weight maintenance are the important goals of obesity treatment which can be done by several ways including the use of lipase inhibitors. Recently, lipase inhibitors from plants such as saponins, polyphenolic compounds and terpenes have garnered increasing attention since they showed sufficient activity (Rahul and Kamlesh, 2007). In order to search for the new sources of lipase inhibitors, the present study investigated lipase inhibitory activity of several Korean resource plants. Free radicals and reactive oxygen species (ROS) can react with biological molecules, leading to cell and tissue injuries and pathological events. The role of free radicals and ROS in the etiology of many chronic diseases has been well known. Therefore, free radical scavengers and antioxidants are important for human health and have been proposed as health promoting natural products. When the mechanism of antioxidant protection becomes unbalanced by factors such as aging, deterioration of physiological functions may occur resulting in diseases and accelerating aging. An example of oxidative stress in disease is observed in diabetes mellitus, which is aggravated by an increase in oxidative stress (Carneiro, 2004). This study was also designed to determine the antioxidant capacities of the selected 11 kinds of resource plants by the DPPH radical scavenging method and study the correlation between α-amylase, α-glucosidase, lipid inhibitory effects and/or antioxidant activity. Recent interests in the study of functional plants have focused on their potential benefits to human health. The functional plants have been used as traditional medicine for the treatment of diabetes, but scientific evaluation is still lacking in this regard. The plants continue to play an important role in the treatment of diabetes and obesity control. The increase in demand to use alternative approaches to treat diabetes and hyperlipidaemia, such as plant-based medicines, is also due to the side effects associated with the use of insulin and oral hypoglycemic agents and anti-obesity agents. Therefore, in this study, we were screening of α-amylase, α-glucosidase, pancreatic lipase inhibitory activities *in vitro*, and DPPH radical scavenging activity from some economic resource plants extracts.

Materials and Methods

Plant material and extract preparation

In this experiment, 11 kinds of plant materials (Salicornia herbacea, Corylopsis coreana, Erythronium japonicum, Phragmites communis, Momordica Charantia, Nelumbo nucifera, Salvia plebeia, Portulaca oleracea, Ficus carica, Citrus junos and Cornus officinalis) of economic resource plants were used. These plants were chosen because of the possibility to obtain various physiological functionalities. Each sample was freeze dried and then ground into a fine powder. Each plant powder was stored at -20℃ for further experiments. Methanol and water extracts were prepared by soaking the sample powder into 100% methanol or distilled water for 24 hours at room temperature. The crude extract was filtered through a whatman filter No. 3.

Measurement of α- amylase inhibitory activity *in vitro*

The α-amylase inhibitory assay was adapted and modified from Satoyama *et al.* (1998) and Oh *et al.* (2010). An identical volume of 2% starch solution (0.1 M citric acid; pH 6.0) and 3.2% agar solution (0.1 M citric acid; pH 6.0) was mixed at 60℃ water bath, and then substrate plate was made by the addition, and cooling of 100 μL in each well of microp-

late. After incubation at 37℃ for 10 min, 25 μL of α-amylase (10 unit /mL) and 25 μL of each plant extract were added in each well and followed by a 120 min reaction at 37℃. The α-amylase activity was determined by measuring the absorbance of the mixture at 655 nm, using a spectrophotometer (Biochrom Co., England). The α-amylase activity (inhibition rate percent) was calculated using the following equation:

$$\alpha\text{-amylase inhibitory activity (\%)} = [1 - (A_i - A_f)/ (B_i - B_f)] \times 100$$

A_i–A_f; the absorbance of reaction solution before and after reaction

B_i–B_f; the absorbance of the blank test before and after reaction

Measurement of ***α*** - glucosidase inhibitory activity *in vitro*

The α-glucosidase inhibitory assay of the extracts was adapted from Kim *et al.* (2011) and Wang *et al.* (2006) with slight modification. The sample extract of 20 mg/mL concentration and 720 μL of 0.2 M potassium phosphate buffer (pH 6.8) containing 100 μL of α-glucosidase solution (0.15 U/mL) were mixed. After measuring the absorbance of the mixture at 405 nm and keeping at room temperature for 5 min, 100 μL of 5 mM 4-nitrophenyl- α-D-glucopyranoside solution was added, more reacted at room temperature for 10 min, and then the absorbance reading of the reaction mixtures were recorded at 405 nm. The α-glucosidase inhibitory activity was calculated from the change in absorbance and was expressed as inhibition %.

$$\alpha\text{- glucosidase inhibitory activity (\%)} = [(\text{Control}_{405} - \text{Extract}_{405})]/\text{Control}_{405} \times 100$$

Measurement of pancreatic lipase inhibitory activity *in vitro*

The inhibitory activity against pancreatic lipase was measured using p-nitrophenyl butyrate (p-NPB) as a substrate with modified method from Zhang *et al.* (2008). The pancreatic lipase stock solution was prepared to a concentration of 1 mg/mL in 0.1 mM potassium phosphate (pH 6.0), and then was stored at -20℃. The assay mixture contained 25 μL of the extracts and 25 μL of the pancreatic lipase solution was added in 950 μL of 0.1 mM potassium phosphate (pH 7.2, 0.1% Tween 80), after incubation at 30°C for 1 hour, and then was reacted at 37°C for 5 min with 1 μL of p-NPB. The lipase inhibitory activity was determined by measuring the absorbance at 405 nm, and was calculated using the following equation:

$$\text{Lipase inhibitory activity (\%)} = [1 - (B - C)/A] \times 100)$$

A: Absorbance of reaction solution without the sample extract

B: Absorbance of reaction solution with the sample extract

C: Absorbance of reaction solution without the enzyme

Measurement of antioxidant activity

The antioxidant activity assay of each extract was performed by the measuring of the electron donor capacity of DPPH. 100 μL of various concentrations (100, 250, 500, 1000 and 2500 mg L^{-1}) of extracts of the extracts of the investigated plants were added to 900 μL of 100% methanol containing 100 μM DPPH, and the reaction mixture was shaken vigorously. After storage at room temperature for 30 min in darkness, the absorbance of DPPH was determined by spectrophotometer at 517 nm. The DPPH radical scavenging activity was calculated according to the following equation: Scavenging effect on DPPH radical (%) = [(A - B)/A] × 100, Where A is the absorbance at 517 nm without pigment compositions and B is the change in absorbance at 517 nm with pigment compositions incubation (Brand-Williams *et al*., 1995).

Data analysis

The statistical analysis was performed using the procedures of the Statistical Analysis System (SAS version 9.1). ANOVA procedure followed by Duncan test was used to determine the significant difference ($p < 0.05$) between treatment means.

Results and Discussion

α-Amylase inhibition

Inhibition of α-amylase by various extracts of Korean

resource plants was shown in Fig. 1. α-Amylase inhibitory activity of *Salicornia herbacea*, *Erythronium japonicum* (flower), and *Phragmites communis* (root) in water extract was shown 62.8%, 66.5% and 69.3%, respectively and the activity of methanol extract was found to have a higher inhibitory effect with 32.8% in *Citrus junos* (pericarp) and 60.9% in *Cornus officinalis* than the other extracts. However, α-amylase inhibitory activity of the methanol extracts in all experiment plants were found to appear significantly lower than its of the water extracts. Management of blood glucose level is a critical strategy in the control of diabetes and its complications (Dicarli *et al*., 2003). α-Amylase and α-glucosidase inhibitors have been useful as oral hypoglycemic

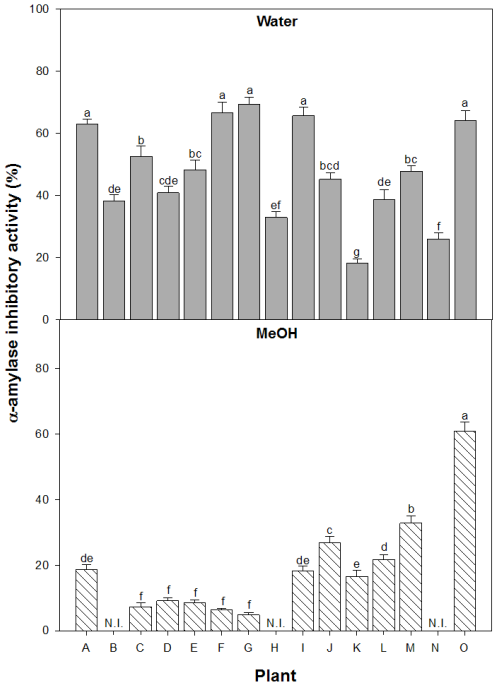

Fig. 1. Inhibition of α-amylase activity by different plants. Means with the same letter in column are not significantly different at $p<0.05$ level by Duncan's multiple range test. The bars represent the standard error. N.I.: Not inhibited. A: *Salicornia herbacea*, B: *Corylopsis coreana* (Stem), C: *Corylopsis coreana* (Flower), D: *Erythronium japonicum* (Leaf), E: *Erythronium japonicum* (Root), F: *Erythronium japonicum* (Flower), G: *Phragmites communis* (Root), H:: *Nelumbo nucifera* (Leaf), I: *Salvia plebeia*, J: *Momordica Charantia*, K: *Portulaca oleracea*, L: *Ficus carica* (Leaf), M: *Citrus junos* (Pericarp), N: *Citrus junos* (Leaf), O: *Cornus officinalis*.

drugs for the control of hyperglycemia especially in patients with type 2 diabetes mellitus. Inhibition of these enzymes delay carbohydrate digestion and prolong overall carbohydrates digestion time causing a reduction in the rate of glucose absorption and consequently reducing postprandial plasma glucose rise (Kimura *et al*, 2006). The α-amylase inhibitory effects of water and methanol extract may be attributed to the presence of phytochemicals. This also explains the reason behind the effective inhibitory activity displayed by the water extract towards the enzyme when compared to that exhibited by methanol extract. α-Amylase is an enzyme found in the salivary, intestinal mucosal and pancreatic secretions, functioning in the breakdown of the α-1-4-glycosidic bonds in starch. Thus, this enzyme increases the bioavailability of glucose in the blood. For a substance to be anti-diabetic, it should be able to reduce the amount of glucose in the blood or increase the efficacy of insulin. It has been reported that the inhibition of α-amylase reduces the bioavailability of glucose, the determination of 40 drugs that inhibit carbohydrate hydrolyzing enzymes have been proved to decrease postprandial hyperglycemia and improve impaired glucose metabolism without promoting insulin secretion in non-insulin dependent diabetes mellitus (NIDDM) patients (Ashok Kumar *et al.,* 2011). Our study demonstrates an appreciable α-amylase inhibitory activity with the water extract of investigated plants. This study endorses the use of these plants for further studies to determine their potential for type 2 diabetes management. In particular, results suggests that extracts of *Salicornia herbacea*, *Erythronium japonicum* and *Phragmites communis* act effectively as α-amylase inhibitors leading to a reduction in starch hydrolysis and hence eventually to lowered glucose levels.

α-Glucosidase inhibition

The measurement results of α-glucosidase inhibitory activity in the 11 kinds of economic resource plants are shown in Fig. 2. *Corylopsis coreana* in both water and methanol extract had the highest α-glucosidase inhibitory activity of 81.7% and 89.5%, while the extract of *Portulaca oleracea*, *Ficus carica* and *Citrus junos* was not measured α-glucosidase inhibitory activity at given experiment concentration. Depending on the extraction solvent and the plant

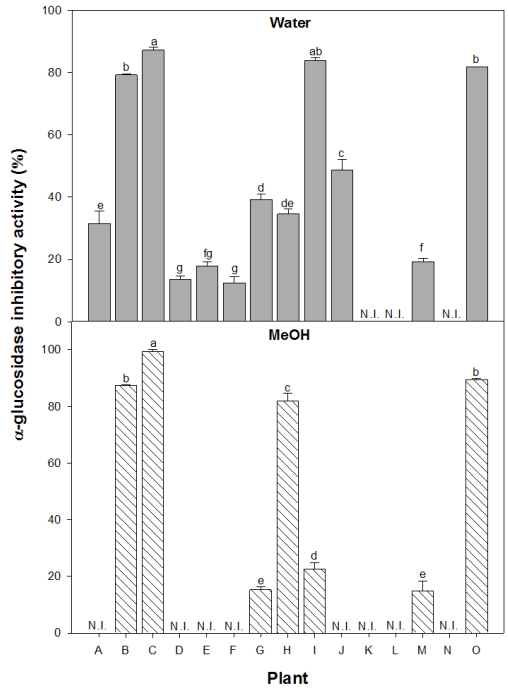

Fig. 2. Inhibition of α-glucosidase activity by different plants. Means with the same letter in column are not significantly different at $p<0.05$ level by Duncan's multiple range test. The bars represent the standard error. N.I.: Not inhibited. A: *Salicornia herbacea*, B: *Corylopsis coreana* (Stem), C: *Corylopsis coreana* (Flower), D: *Erythronium japonicum* (Leaf), E: *Erythronium japonicum* (Root), F: *Erythronium japonicum* (Flower), G: *Phragmites communis* (Root), H:: *Nelumbo nucifera* (Leaf), I: *Salvia plebeia*, J: *Momordica Charantia*, K: *Portulaca oleracea*, L: *Ficus carica* (Leaf), M: *Citrus junos* (Pericarp), N: *Citrus junos* (Leaf), O: *Cornus officinalis*.

species, it was observed that there was a significant difference in α-glucosidase inhibitory activity. α-Glucosidase, a key enzyme for carbohydrate digestion, has been recognized as a therapeutic target for modulation of postprandial hyperglycemia, which is the earliest metabolic abnormality to occur in type 2 diabetes mellitus (Vadivelan *et al.,* 2012). Many natural resources have been investigated with respect to suppression of glucose production from carbohydrates in the gut or glucose absorption from the intestine (Hara and Honda, 1990). α-Amylase catalyses the hydrolysis of a-1, 4-glucosidic linkages of starch, glycogen and various oligosaccharides and α–glucosidase further breaks down the disaccharides into simpler sugars, readily available for the intestinal absorption. The inhibition of their activity, in the digestive tract of humans, is considered to be effective to control diabetes by diminishing the absorption of glucose decomposed from starch by these enzymes (He *et al.,* 2006). Therefore, effective and nontoxic inhibitors of a-amylase and a-glucosidase have long been sought. Our present research suggest that the presence of potential functional compounds of *Corylopsis coreana, Nelumbo nucifera*, *Salvia plebeian* and *Cornus officinalis* may have a potentially important role in managing diabetes via the inhibition of α-glucosidase enzyme activity. The extracts of these plants have the advantage of having α-glucosidase inhibitor action hence could prove to be an effective treatment for diabetes mellitus.

Pancreatic lipase inhibition

In this study, the inhibitory effect of 15 extracts from 11 Korean resource plants against pancreatic lipase was investigated (Fig. 3). The pancreatic lipase inhibitory activity of methanol extract was found to have a higher the effect than water extract. Inhibition of lipase activity of the *Erythronium japonicum* (flower), *Salvia plebeia*, *Citrus junos* (pericarp) and *Cornus officinalis* showed a relatively high from 59.3% to 75.1%. The inhibitory activity of water extract showed 45.8% in *Corylopsis coreana* (flower) and 83.3% in *Citrus junos* (pericarp). However, unlike the results of a-amylase and a-glucosidase inhibitory activity, pancreatic lipase inhibitory activity showed relatively high in the methanol extract than water extract except pericarp of *Citrus junos.* Obesity is an increasingly serious global problem, not only for the harm it causes in its own right, but also due to the associated health threats, especially type 2 diabetes, systemic hypertension, cardiovascular disease, certain cancers, asthma, and sleep apnea. (Kopelman, 2000; Finer, 2006). The prevalence of obesity has been steadily increasing in children and adolescents in recent years (Mokdad *et al.,* 2001; Styne, 2001), which suggests the likelihood of worsening obesity trends in the future adult population. Consumption of edible plants could be a more effective method for the prevention or treatment of hyperlipidaemia. Many edible plants present an exciting opportunity for the development of newer therapeutics for biologically active antihyperlipidaemic agents from natural resources, especially the reduction of fat digestion

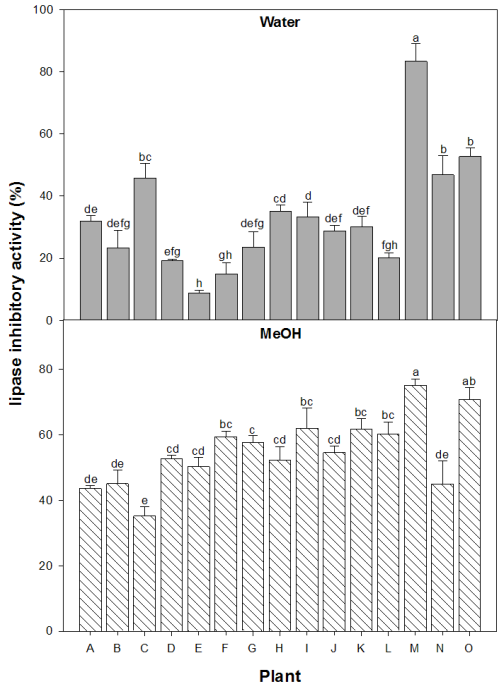

Fig. 3. Inhibition of lipase activity by different plants. Means with the same letter in column are not significantly different at $p<0.05$ level by Duncan's multiple range test. The bars represent the standard error. A: *Salicornia herbacea*, B: *Corylopsis coreana* (Stem), C: *Corylopsis coreana* (Flower), D: *Erythronium japonicum* (Leaf), E: *Erythronium japonicum* (Root), F: *Erythronium japonicum* (Flower), G: *Phragmites communis* (Root), H: *Nelumbo nucifera* (Leaf), I: *Salvia plebeia*, J: *Momordica Charantia*, K: *Portulaca oleracea*, L: *Ficus carica* (Leaf), M: *Citrus junos* (Pericarp), N: *Citrus junos* (Leaf), O: *Cornus officinalis*.

and absorption (Kurihara *et al.,* 2006; Zhang *et al.,* 2008; Moller *et al.,* 2009). Pancreatic lipase inhibitors which help to limit intestinal fat absorbtion at the initial stage, have been proved as useful medications for the treatment of hyperlipidaemia and a great promise as anti-obesity agents (Sharma *et al.,* 2005). The mechanism of lipase inhibitors is to block the absorption of fat by inhibiting lipase in the gastrointestinal tract (Davidson, *et al.,* 1999). The wildly prescribed of lipase inhibitors, orlistat, has been shown to benefit in weight control in obesity. However, orlistat induces the gastrointestinal side effects which may cause the premature withdrawals (Sjöström *et al.,* 1998). Consequently, some Korean resource plants in this study may have great potential as dietary supplements or nutraceutical foods for lipase inhibitory effect with antihyperlipidaemic properties.

DPPH radical scavenging activity

The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability. The investigation of the antioxidant activity of natural substances is based on the measuring of the electron donor capacity of DPPH with the ability to inhibit the oxidation by donating electrons in free radicals causing this lipid peroxidation (Boo *et al.,* 2012). Free radicals are known to be a major factor in biological damages, and DPPH has been used to evaluate the free radical-scavenging activity of natural antioxidants (Yokozawa *et al.,* 1998; Zhu *et al.,* 2001). Active oxygen caused by in vivo metabolism removed by the body's antioxidant system, but excessive free radicals induced stress, causing the lipid peroxidation by combining with unsaturated fatty acids in the cell membrane, and brought intracellular structural and functional damage. DPPH; which is a radical itself with a purple color, changes in to a stable compound with a yellow color by reacting with an antioxidant and the extent of the reaction depends on the hydrogen donating ability of the antioxidant (Korycks-Dahl and Richardson, 1977). The reduction capability of the DPPH radical is determined by the decrease in its absorbance at 517 nm, induced by antioxidants. The measurement results of free radical scavenging activity at seven different concentrations, 100, 250, 500, 1000 and 2500 mg/L are shown in Table 1. The extracts of *Corylopsis coreana*, *Salvia plebeia* and *Cornus officinalis* showed strong radical scavenging activity (Table 1). The DPPH radical scavenging activity of selected plants was much difference between measured plant species, and showed that the increase was proportional to the concentration. This study suggests that some functional substrates involved in radical scavenging activities may be involved in the α-amylase inhibition. The chemical composition, antioxidant capacity and α-amylase inhibitory activity of both *Corylopsis coreana* and *Cornus officinalis* possess antioxidant property and can potentially play an important role in controlling diabetic. Additional studies in this point are required to evaluate their potential in control of sugar levels in diabetic patients and as a free radicals scavenging.

Table 1. DPPH radical scavenging activities according to each kind of several economic resource plants

Plants	DPPH radical scavenging activity, % of control				
	Concentration (mg/L)				
	100	250	500	1000	2500
Salicornia herbacea	ND	ND	6.7±0.32e	14.2±1.12ef	22.8±1.52ef
Corylopsis coreana(Stem)	2.8±0.16c	5.1±0.21d	12.3±1.02d	35.8±1.39b	53.9±1.29c
Corylopsis coreana(Flower)	4.9±0.28b	9.8±0.65b	17.5±0.83b	30.6±0.95c	70.1±1.35b
Erythronium japonicum(Leaf)	ND	2.2±0.13f	4.2±0.17f	8.3±0.73gh	19.1±0.78fg
Erythronium japonicum(Root)	ND	ND	ND	3.1±0.17i	6.6±0.52i
Erythronium japonicum(Flower)	ND	ND	1.2±0.06gh	3.7±0.32i	18.5±1.05g
Phragmites communis(Root)	ND	ND	ND	2.6±0.16i	6.5±0.76i
Nelumbo nucifera(Leaf)	ND	3.9±0.08e	7.1±0.78e	16.9±0.38e	41.2±2.06d
Salvia plebeia	5.6±0.35a	8.6±0.31c	14.8±1.06c	24.2±1.21d	71.5±1.83b
Momordica Charantia	ND	2.8±0.12f	7.2±0.25e	9.5±1.08g	23.7±1.95e
Portulaca oleracea	ND	ND	0.8±0.07gh	5.5±0.32hi	14.1±1.16h
Ficus carica(Leaf)	ND	ND	2.8±0.19fg	5.1±0.27i	7.8±0.62i
Citrus junos(Pericarp)	ND	2.9±0.27f	7.5±0.15e	11.4±0.25fg	18.6±0.81g
Citrus junos(Leaf)	ND	0.8±0.05g	2.3±0.09fgh	4.3±0.38i	14.2±0.17h
Cornus officinalis	5.8±0.72a	11.5±0.73a	31.2±2.25a	66.3±2.82a	81.2±1.92a

[z]Data represent the mean values±SE of three independent experiments. Means with the same letter in column are not significantly different at $p<0.05$ level by Duncan's multiple range test.
N.D.: Not detected

In conclusion, this study investigated the potential anti-diabetic and anti-obesity activity of selected Korean resource plants, focusing on the inhibitory effects on α –amylase, α-glucosidase and pancreatic lipase *in vitro*. We conjectured that the selected plants had the potent biological activity on carbohydrate, lipid Inhibitory activity and antioxidant activity, therefore these plant resources could be a good materials to develop medicinal preparations, nutraceuticals or health functional foods for diabetes or obesity and related symptoms.

Acknowledgement

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