ejpmr, 2019,6(11), 110-114

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article
ISSN 2394-3211
EJPMR

EVALUATION OF ALPHA-AMYLASE AND ALPHA-GLUCOSIDAE INHIBITORY POTENTIAL OF *SEDUM SARMENTOSUM*

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Article Received on 14/09/2019 **Article Revised on 04/10/2019** **Article Accepted on 25/10/2019**

ABSTRACT
The purpose of present study was to investigate the effects of extracts of *Sedum sarmentosum* a traditional vegetative plant, on α-amylase and α-glucosidase activities *in vitro.* The three tissues (leaves, stems, and roots) of *S. sarmentosum* were extracted with distilled water (AE) and aqueous methanol (MeOH-H_2O) were obtained from MeOH-H_2O crude extracts per liquid/liquid extraction. The results of the both enzyme inhibition activity was found in a dose-dependent manner. The high α-amylase inhibitory found on leaves extracts. α-amylase inhibition effects of leaves of *S. sarmentosum*evaluated at 1.0 μg/ml of distilled water extract (DWE) was 29.4% and that of stems was 25.1% at same concentration. The values of α-amylase IC_{50} for leaves with DWE and ETE were 15.11 μg/ml and 16.08 μg/ml, respectively. Those of α-glucosidase IC_{50} for leaves with DWE and ETE were 14.55 μg/ml and 13.64 μg/ml, respectively. α-glucosidase inhibition for DWE of leaves evaluated at 1.0 μg/ml was 50.5% and that of stems was 39.7% at same concentration. All extract from this plant possess moderate α-amylase inhibition with potent α-glucosidase inhibitory activity.

KEYWORDS: α-amylase, α-glucosidase, *Sedum sarmentosum.*

INTRODUCTION
Sedum sarmentosum Bunge (Family Crassulaceae) has succulent, evergreen leaves atop arching, low-lying stems up to 10 inches (25 cm) long. The fresh leaves are arranged in whorls of 3 at intervals along the stem. *S. sarmentosum* is native to East Asia (China and Korea) and Southeast Asia (Thailand).[1] In Korea, the plant is called dolnamul and is eaten fresh as a namul vegetable. *S. sarmentosum* also known as stonecrop (dolnamul) is a widely consumed herb and is used as an ingredient in salads in Korea.[2] *S. sarmentosum* extract has been used traditionally to treat liver inflammatory diseases in the Asian area.[3] *S. sarmentosum* extract contains multiple active flavonoids (such as quercetin, isorhamnetin and kaempferide),[4-6] has marked renal anti-fibrotic effects.[7,8] The methanol-eluted fraction of the hot water extract from the whole plant of *S. sarmentosum* was found to show hepatoprotective effect on D-galactosamine-induced cytotoxicity in primary cultured mouse hepatocytes.[5] Bioassay-guided fractionation of the EtOAc-soluble extract of *Sedum sarmentosum* afforded a new flavonoid, quercetin-3-O-alpha-(6'''-caffeoylglucosyl-beta-1,2-rhamnoside), along with four known flavonoids, quercetin 3-O-alpha-(6'''-p-coumaroylglucosyl-beta-1,2-rhamnoside), isorhamnetin-3-beta-glucopyranoside, quercetin-3-beta-glucopyranoside, and kaempferol-3-alpha-arabinopyranoside.[8] *S. sarmentosum* extract had protective effects on anti-inflammation, antiaging, protecting the liver, reducing aminotransferase, antitumor, antihepatic fibrosis, and promoting the regeneration of liver cells.[9-12] Flavanones from *S. sarmentosum* are the major bioactive ingredient derived from the herbal SSB, which contains quercetin, luteolin, kaempferol, and isorhamnetin.[13]

Small internal alpha (α)-glucosidase (EC 3.2.1.20) and pancreatic α-amylase (EC 3.2.1.1) are key enzymes of diary carbohydrate digestion in humans.[14] Inhibition of α-glucosidase and α-amylase, enzymes involved in the digestion of carbohydrates, can significantly decrease the postprandial increase of blood glucose after a mixed carbohydrate diet and therefore can be an important strategy in the management of postprandial blood glucose level in type 2 diabetic patients and borderline patients.[15] α-amylases catalyze the hydrolysis of internal alpha-1,4-glucosidic linkages in starch and other related polysaccharides and have also been target for suppression of postprandial hyperglycemia.[16] Inhibition of these enzymes can retard carbohydrate digestion, thuscausing a reduction in the rate of glucose absorption into the blood. Therefore, inhibition of these enzyme activities in digestive organs is considered to be a therapeutic approach for managing diabetes.

In this paper we compare the inhibition of mammalian α-amylase and α-glucosidase by different tissues of *S. sarmentosum* so as to get a better understanding of their contribution to inhibitory activities in plant foods or herbs.

MATERIALS AND METHODS

Sample extract

The plants of *S. sarmentosum* divided into three parts: leaves, stems, and roots. Each sample (1 kg) was small pieces of crushed with a grinding mixer. Because roots are light weight, they used only 500 g and cut the extract buffer in half. They were squeezed out with the muslin cloth and was putin 1000 mL beaker. The samples were blended with 80% ethanol or distilled water, and then an aliquot of the mixture (80% ethanol or distilled water) was further mixed with 100 mM Tris-HCl buffer (400 µL, pH 7.4). The mixture was further stirred with a magnetic bar at 65°C for 12 hours. The sample was treated with ultrasound at room temperature for a given duration. The ultrasound extraction was carried out using an ultrasonic bath (5510, Branson, USA). The mixture was shaken vigorously for one hour at roomtemperature and left in the dark at room temperature for 20 min. Extracted sample was filtered. The samplewas evaporated to remove solvent under reduced pressure and controlled temperature by using rotary vacuum evaporator (N-1001S-W, Eyela, Tokyo, Japan). To get dry powder, samples placed in a low temperature vacuum chamber.

α-amylase inhibitory assay

α-amylase inhibitory activity was determined by the method described Apostolidis[17] with some modification. 2.5 µl extract and 25 µl of 50 mM phosphate buffer pH 6.8, containing porcine α-amylase (0.25 U/ml) were incubated at 37°C for 10 min. After pre incubation, 25 µl of 0.5% starch solution was added. The reaction mixtures were then incubated at 37°C for 10 min. The reaction was terminated with the addition of 150 µl of 90 mM 3,5-dinitrosalicylic acid (DNS) reagent and placed in boiling water bath for 10 minutes. The extract was then cooled to room temperature until use. Acarbose (4",6"-Dideoxy-4"-([1S]-[1,4,6/5]-4,5,6-trihydroxy-3-hydroxymethyl-2-yclohexenylamino)-maltotriose) (Sigma Aldrich Chemical Co, USA) was used as reference standard (positive control). Control incubations represent 100% enzyme activity and were conducted in a similar way by replacing extracts with vehicle. For blank incubation (toallow for absorbance produced by the extract), enzyme solution was replaced by buffer solution and absorbance recorded. Separate incubation carried out for reaction t = 0 was performed by adding samples to DNS solution immediately after addition of the enzyme. Absorbance (A) was measured at 540 nm. The concentration of the extract required to inhibit the activity of the enzyme by 50% (IC_{50}) was calculated by regression analysis. Experiments were performed in duplicate.

Percent α-amylase inhibition was calculated as follows: (1–B/A) × 100, where A is the absorbance of control and B is the absorbance of samples containing extracts.

α-glucosidase inhibitory assay

The α-glucosidase inhibition assay was adapted from Deutschlander et al.[18] with some modification. Extracts and catechins were prepared as described above. The test compound and 2 mU of α-glucosidase (Sigma Aldrich Chemical Co, USA) from *S. sarmentosum*, were diluted to 97 µL in 0.1 M sodium acetate buffer (pH 5.6) and pre-incubated in 96-well plates at 37°C for 10 min. The reaction was initiated by adding 3 µL of 3 mM pNPG as substrate. The plate was incubated for an additional 10 min at 60°C, followed by addition of 100 µL 1 M NaOH to stop the reaction. All test compounds were prepared in DMSO as described above. The final concentrations of extracts and catechins were between 0.03–10 µg/mL and 5–1000 µM, respectively. The final concentration of α-glucosidase was 20mU/mL. The optical density (OD) of the solution was read using the Microplate Reader (VersaMax, Califonia, USA) at the wavelength 410 nm.

The inhibitory concentration of the extract required to inhibit the activity of the enzyme by 50% (IC_{50}) was calculated by regression analysis. Experiments were performed in duplicate. Acarbose was usedas reference standard (positive control). Acarbose, known as BAY g 5421, is an α-glucosidase inhibitor that prevents absorption of sucrose and maltose.

Inhibition of free radical scavenging activity was calculated using the following equation.

Inhibition (%) = 100 × (absorbance of the control - absorbance of the sample)/ absorbance of the control.

The ability of the extracts to scavenge at 50% of the α-amylase and α-glucosidase, IC_{50} was determined from the graph plotted in Graph Pad Prism software. Regression analysis by a dose response curve was plotted to determine the IC_{50} values.

Statistical Analyses

All assays were carried out in triplicate and the values presented are the average of 3 replicates. The results were expressed as the mean±SD. Statistical analyses of the differences between samples were carried out by one-way analysis of variance (ANOVA), followed by post hoc multiple comparisons with Duncan's test and student's t-test with the PASW (Predictive analytics software) statistics package for Windows program. Differences were considered significant if the *p*-value was less than 0.05.

RESULTS

α-amylase inhibitory effects

The *in vitro* α-amylase inhibitory studies demonstrated that *S. sarmentosum* had α-amylase inhibitory activity. The percentageinhibition at 0.1, 0.5, 1.0 µg/ml concentrations. *S. sarmentosum* extract showed a concentration-dependent reduction in percentage

inhibition. Thus the highest concentration of 1.0 µg/ml tested showed a maximum inhibition of nearly 50.1%. Table 1 was shownthe inhibition effects for α-amylase of *S. sarmentosum.* The high α-amylase inhibitory found on leaves extracts. α-amylase inhibition effects of leaves of *S. sarmentosum* evaluated at 1.0 µg/ml of distilled water extract (DWE) was 29.4% and that of stems was 25.1% at same concentration. The rates of α-amylase inhibition of the ethanol extract (ETE) for *S. sarmentosum* were also dependent on concentrations. α-amylase inhibition for ETE of leaves evaluated at 1.0 µg/ml was 50.1% and that of stems was 29.1% at same concentration. α-amylase inhibition effects of roots of *S. sarmentosum* evaluated at 1.0 µg/ml of distilled water extract (DWE) was 13.3% and that of ETE was 17.2% at same concentration. Although the rates of α-amylase inhibition of the ethanol extract (ETE) for *S. sarmentosum* were also dependent on concentrations, there werenot shown a statistically significant difference among groups (three tissues) and between groups (DWE and ETE) ($p < 0.05$). However, inhibitory activities for α-amylase of *S. sarmentosum* showed a statistically significant difference between three concentration groups ($p < 0.05$).

Figure 1was shown the rate of α-amylase inhibitory of Acarbose (positive control) and relative inhibitory rate for three tissues of *S. sarmentosum.* The values for leaves with DWE and ETE were 30.4% and 51.9% on 1.0 µg/ml, respectively. The values for stems with DWE and ETE were 26.0% and 30.1%, respectively. The values for roots with DWE and ETE were 13.8% and 17.8%, respectively.

Table 1: The degree of inhibition (%) of α-amylase by tissues of *Sedum sarmentosum* at different concentrations.

Type	Concentration (µg/ml)	Extract		*t*-test
		DWE	ETE	
Leaves	0.1	5.88±0.91	10.30±0.14	2.262
	0.5	15.46±0.25	23.16±2.54	
	1.0	29.38±2.88	50.13±2.92	
Stems	0.1	4.09±1.32	5.51±1.04	0.238
	0.5	14.89±3.38	16.04±2.76	
	1.0	25.14±1.06	29.09±2.93	
Roots	0.1	1.09±0.62	3.30±0.89	0.631
	0.5	5.03±2.10	9.16±1.68	
	1.0	13.29±4.33	17.16±1.11	
F-test		1.569	0.542	

Data represented the mean ± SD from three replicates. DWE: distilled water extract, ETE: ethanol extract.

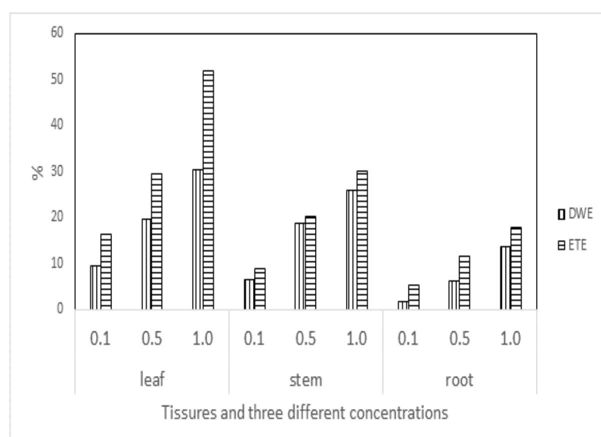

Figure 1: The Rate of α-Amylase Inhibitory of Acarbose (Positive Control) and Relative Inhibitory Rate For Tissues of *Sedum Sarmentosum* on Three Different Concentrations (0.1, 0.5, and 1.0 M).

α-glucosidase inhibitory effects

The results of the α-glucosidase inhibitory effects of DWE and ETE of *S. sarmentosum* in comparison with the standard (Acarbose) at 410 nm were shown in Table 2. It was observed that inhibition percentage values go on increasing with enhancements in concentration of research plant extracts in the assay mixture. α-glucosidase inhibition for DWE of leaves evaluated at 1.0 µg/ml was 50.5% and that of stems was 39.7% at same concentration. α-glucosidase inhibition for ETE of leaves evaluated at 1.0 µg/ml was 62.6% and that of stems was 42.3% at same concentration. α-glucosidase inhibition effects of roots of *S. sarmentosum* evaluated at 1.0 µg/ml of distilled water extract (DWE) was 22.2% and that of ETE was 27.6% at same concentration. The all values of α--glucosidase inhibitory for ETE of *S. sarmentosum* were higher than those of DWE. The all groups (three tissues) showed a statistically significant difference ($p>0.05$).

Figure 2 was shown the rate of α-amylase inhibitory of Acarbose (positive control) and relative inhibitory rate for three tissues of *S. sarmentosum*. The values for leaves with DWE and ETE were 52.3% and 64.8% on 1.0 µg/ml, respectively.

An IC50 value is the concentration of the extract required to inhibit the activity of the enzyme by 50% of the free radicals present in the system. IC50 value was inversely related to the antioxidant activity of crude extracts. The values of α-amylase IC50 for leaves with DWE and ETE were 15.11µg/ml and 16.08µg/ml, respectively (Table 3). Those of α-glycosidase IC50 for leaves with DWE and ETE were 14.55µg/ml and 13.64µg/ml, respectively.

Table 2: The Degree of Inhibition (%) of α-glucosidase by Tissues of *Sedum Sarmentosum* at Different Concentrations.

Type	Concentration (μg/ml)	Extract		*t*-test
		DWE	ETE	
Leaves	0.1	13.73±1.31	18.73±2.13	0.526
	0.5	31.06±2.64	40.04±1.88	
	1.0	50.51±0.91	62.60±2.68	
Stems	0.1	10.80±0.56	12.70±2.59	0.582
	0.5	26.14±3.05	24.65±3.69	
	1.0	39.68±4.73	42.28±3.93	
Roots	0.1	6.62±1.55	8.58±2.10	0.247
	0.5	14.61±0.88	19.74±3.31	
	1.0	22.24±1.66	27.60±2.46	
F-test		1.887	1.582	

Data represented the mean ± SD from three replicates. DWE: distilled water extract, ETE: ethanol extract.

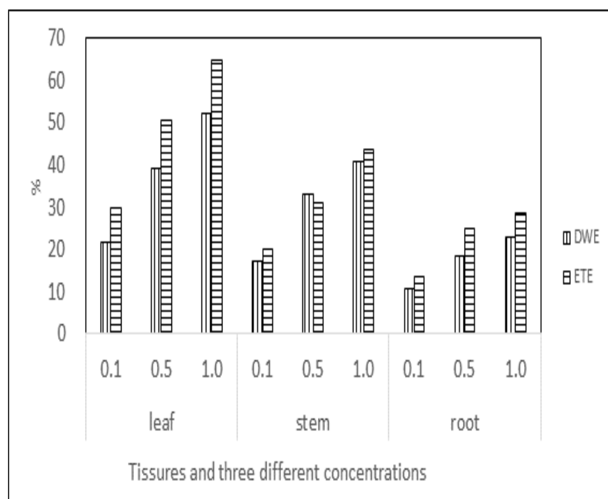

Figure 2. The Rate of α-glucosidase Inhibitory of Acarbose (Positive Control) and Relative Inhibitory Rate For Tissues of *Sedum Sarmentosum* on Three Different Concentrations (0.1, 0.5, And 1.0 M).

Table 3: The 50% Inhibition (IC_{50}) of α-Amylase and α-glucosidase of *Sedum Sarmentosum* at Different Tissues and Solvents.

Sample	α-amylase		α-glucosidase	
	Water	Ethanol	Water	Ethanol
Leaves	16.11	15.08	14.55	13.64
Stems	16.47	16.18	15.13	14.87
Roots	17.40	17.05	16.51	16.03

DISCUSSION

Ethanol extract showed more high α-amylase and α-glucosidase inhibitory activity than that of water extract (Table 1). Although *S. sarmentosum* has not inhibited alpha-glucosidase more potently than the standard Acarbose, it was observed that inhibition percentage values go on increasing with enhancements in concentration of research plant extracts in α-glucosidase inhibition. However, extracts of *S. sarmentosum* did not show any effect on alpha-amylase enzyme. Similar result has been reported by Kwon et al.[19] (inhibition of alpha-glucosidase enzyme, but no inhibitory activity on alpha-amylase enzyme by clonal extracts of herbs of Lamiaceae species). IC50 of ethanol extract of *S. sarmentosum* was 13.6 μg/ml (Table 3).

Ethanol extract of *Rhizophora mucronata* showed the highest α-glucosidase inhibitory activity with IC50 value of 9.45 μg/ml.[20] According to Mogale et al.[21], natural inhibitors from plants are reported to have lower inhibitory effect against alpha-amylase and stronger inhibitory activity against alpha-glucosidase and our study supports this finding.

Inhibitors of α-glucosidase delay the breaking down of carbohydrate in the small intestine and diminish the postprandial blood glucose excursion in a person suffering from diabetes.[22] One of the strategies and methods adopted to cure diabetes mellitus involves the inhibition of carbohydrate digesting enzymes such as α-amylase and α-glucosidase in the gastrointestinal glucose absorption thereby lowering postprandial glucose level.[23]

The results of the present study would not certainly help to ascertain the potency of the crude extracts in a short time from *S. sarmentosum* as potential source of natural antioxidant.[24] *S. sarmentosum* can be valuable in treatment of diabetes not only through inhibition of α-amylase and α-glucosidase, but also by its antioxidant effect.

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