Antioxidant and α-Glucosidase Inhibitory Activities of the Extract from *Sparganium stoloniferum* Buch.-Ham. Root and Its Constituent Compounds

‒ Research Note ‒

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Abstract

Three compounds, vanillic acid, *p*-hydroxylcinnamic acid, *p*-hydroxybenzaldehyde have been isolated from the ethylacetate extract of *Sparganium stoloniferum* Buch.-Ham roots using silica gel open column chromatography, preparative thin-layer chromatography (pTLC) and reverse phase high performance liquid chromatography. The structures of the compounds were established on the basis of IR, extensive 1D NMR, and MS analyses. The ethylacetate (EtOAc) extract, vanillic acid, and *p*-hydroxybenzaldehyde showed α-glucosidase inhibition activity of 72.71%, 20.13%, and 30.42%, at the concentration of 10 μg/mL, respectively. The EtOAc extract exhibited strong antioxidant activity with an IC_{50} value of 24.37 μg/mL against DPPH radical scavenging activity, the vanillic acid, *p*-hydroxylcinnamic acid, and *p*-hydroxybenzaldehyde with an IC_{50} value of 2.10 μM, 1.59 μM, and 2.72 μM against DPPH, respectively.

Key words: α-glucosidase inhibition, DPPH, *p*-hydroxylcinnamic acid, *p*-hydroxybenzaldehyde, vanillic acid

INTRODUCTION

Sparganium stoloniferum Buch.-Ham. is a perennial wet habitat plant and is widely distributed in East Asia. *S. stoloniferum* has been used as an emmenagogue, a galactagogue, and an antispasmodic agent (1). It has other bioactivities, including anti-platelet aggregation, anti-thrombosis, analgesic, and antitumor (2).

The research described in this study investigated other potential activities of *S. stoloniferum*: anti-diabetic (through α-glucosidase inhibition) and antioxidant. α-Glucosidase inhibitors prevent release of glucose from oligo-saccharides and thereby reduce the postprandial glucose levels and insulin responses (3). Some inhibitors, including acarbose and voglibose are currently used clinically in combination with either diet or other anti-diabetic agents to control blood glucose levels of patients (4). Glycosidase inhibitors have the effects on inhibition of tumor metastasis and inhibition of viral replication (5).

Evaluation of the anti-diabetic and antioxidant properties of the extract of *S. stoloniferum* could be of great interest for both pharmaceutical and functional food purposes. In the present study, we first aimed to isolate some compounds from *S. stoloniferum* that could help combat diabetes via α-glucosidase inhibition activity. We then attempted to isolate any constituent compounds which might be responsible for antioxidant activity.

MATERIALS AND METHODS

Plant materials and chemicals

Sparganium stoloniferum were collected from Jiaozuo, Henan province, China in May, 2008. α-glucosidase, type IV from *S. stearothermophilus*, p-NPG (4-nitrophenyl-α-D-glucopyranoside), DPPH (1,1-diphenyl-2-picrylhydrazyl), L-ascorbic acid were purchased from Sigma (St. Louis, MO, USA).

Extraction and isolation of bioactive compounds

The dried roots of *Sparganium stoloniferum* (5 kg) were extracted twice with MeOH (13 L) at 60°C for 12 hr and then filtered through filter paper (100 mm; Whatman, Maidstone, UK). The methanol filtrates were collected and concentrated under reduced pressure by a rotary evaporator (CCA-1110; EYELA, Tokyo, Japan) to yield 210.6 g extract (SS-M). The extract was suspended in water (0.6 L) and then partitioned with *n*-hexane, CHCl₃, EtOAc, and water-saturated *n*-butanol, repeated 3 times with each solvent. Removal of the solvents afforded 22.0 g, 3.0 g, 3.9 g, 44.5 g, and 137.0 g of the *n*-hexane (SS-M-H), CHCl₃ (SS-M-C), EtOAc (SS-M-EA), *n*-butanol (SS-M-B), and water fractions (SS-M-W), respectively. The α-glucosidase inhibition activity of all the extracts was estimated, with the EtOAc fraction showing the strongest α-glucosidase inhibition activity. This active EtOAc faction (3.9 g) was loaded

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on a silica gel column (5×70 cm, silica gel 230～400 mesh) and eluted with a solvent mixture of $CHCl_3$-EtOAc (gradient from $CHCl_3$ 100% to EtOAc 100%) and then EtOAc-MeOH (gradient from EtOAc 100% to MeOH 100%), affording 12 major subfractions (SS-M-EA1−12). TLC analysis was performed and compounds were visualized under UV light (254 and 365 nm) or by spraying the plates with anisaldehyde-sulfuric acid reagent. The subfractions SS-M-EA3 (1.2 g), SS-M-EA4 (110 mg) were chromatographed on a reverse phase column (2.0×60 cm, LiChroprep RP-18) with MeOH-H_2O (gradient from 30 to 100% MeOH) eluent, affording compounds **1** (13 mg), **2** (16 mg) and **3** (34 mg), respectively. These compounds were further purified by silica gel preparative TLC (pTLC) with CH_2Cl_2-MeOH (97:3) and reverse phase HPLC (Shimadzu LC-6AD, Japan) with CH_3CN-H_2O (65:35) solvent systems. All the compounds were identified by comparing spectral data (UV, NMR) (DMX-300, 400 MHz, Bruker) with reported values.

α-glucosidase inhibition activity

The inhibitory activity against α-glucosidase was measured as described previously (6), with a slight modification. To each well of a 96-well microtiter plate was added 50 μL of α-glucosidase [0.15 U/mL in phosphate buffer (PBS), pH 6.8], 50 μL of PBS (pH 6.8), 50 μL of the sample (concentrations: 1000 μg/mL, 500 μg/mL, 100 μg/mL, 50 μg/mL, 10 μg/mL). The mixture was pre-incubated at 37°C for 15 min, then p-NPG (100 μL, 3 mM in PBS) was added. The mixture was incubated for additional 10 min, then Na_2CO_3 (0.1 M, 750 μL) were added to stop the reaction. The absorbance of each well was measured at 405 nm with microplate spectrophotometer (Bio-Tek, Winooski, VT, USA). The inhibition activity was calculated by the equation:

$$\text{Inhibition (\%)} = [1 - (A_{sample} - A_{blank})/A_{control}] \times 100$$

Where the A_{sample} was the absorbance of the test sample, A_{blank} was the absorbance of the sample (without the substrate), $A_{control}$ was the absorbance of the control (without sample).

The positive control, acarbose (Bayer), was found to have the IC_{50} value of 0.043 μM against the same enzyme.

DPPH radical scavenging activity

The DPPH radical scavenging effects were carried out according to previously published methods (7), with slight modification. Briefly, 0.1 mL of 0.2 mM DPPH in methanol mixed with 0.1 mL compound at various concentrations. The mixture was vortexed and then left

at room temperature for 30 min in the dark. The absorbance was then measured at 517 nm. The ability to scavenge the DPPH radical was calculated using the follow equation:

$$\text{Scavenging effect (\%)} = \left(1 - \frac{A_{sample} - A_{blank}}{A_{control}} \right) \times 100$$

Where the $A_{control}$ is the absorbance of the control (DPPH solution without sample), the A_{sample} is the absorbance of the test sample (DPPH solution and sample), and the A_{blank} is the absorbance of the sample only (sample without DPPH solution). Synthetic antioxidants, L-Ascorbic acid were used as positive controls.

RESULTS

Structural determination of active compounds

The methanol extract of the roots of *S. stoloniferum* was partitioned by different solvents, resulting in layers of n-hexane, $CHCl_3$, EtOAc, n-BuOH and water. The EtOAc fraction was repeatedly subjected to normal-phase silica gel and reverse phase gel chromatography to afford three known phenolic compounds (**1-3**). Their structures were determined by comparing their ^{1}H- and ^{13}C-NMR spectra, and MS data with published values.

Compound **1** was obtained as colorless needle crystal, m.p. 209～211°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.80 (3H, s), 6.84 (1H, d, J=8.7 Hz), 7.44 (1H, brs), 742 (1H, brd, J=8.7 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.9 (COOH), 152.8 (C-4), 148.2 (C-3), 124.8 (C-1), 116.0 (C-2), 113.6 (C-5), 55.6 (OCH_3). ^{1}H-NMR and ^{13}C-NMR data were in close agreement with other reports (8), and the positive FAB-MS m/z 169 [M+H]$^+$ compound was identified as vanillic acid.

Compound **2** was isolated as colorless needle crystal, m.p. 215～216°C. ^{1}H-NMR (400 MHz, pyridine-d_5) δ 7.63 (1H, d, J=15.6 Hz), 6.36 (1H, d, J=15.6 Hz), 7.56 (2H, d, J=8.7 Hz), 6.91 (2H, d, J=8.7 Hz); ^{13}C-NMR (100 MHz, pyridine-d_5) δ 168.9 (C=O), 152.6 (C-4), 148.2 (C-β), 124.8 (C-1), 116.0 (C-α), 115.6 (C-3,5), 113.6 (C-2,6), ^{1}H-NMR and ^{13}C-NMR data are in close agreement with published reports (9), positive FAB-MS m/z 165 [M+H]$^+$, so this compound was identified as *p*-hydroxylcinnamic acid.

Compound **3** was obtained as white crystal, m.p. 115～117°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.82 (2H, d, J=8.7 Hz, 2-H and 6-H), 7.05 (2H, d, J=8.7 Hz, 3-H and 5-H), 9.86 (1H, s, 7-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 191.0 (C-7), 163.2 (C-4), 132.3 (C-2,6), 124.7 (C-1), 115.6 (C-3 and 5). ^{1}H-NMR and ^{13}C-NMR data are in close agreement with previous reports (10), pos-
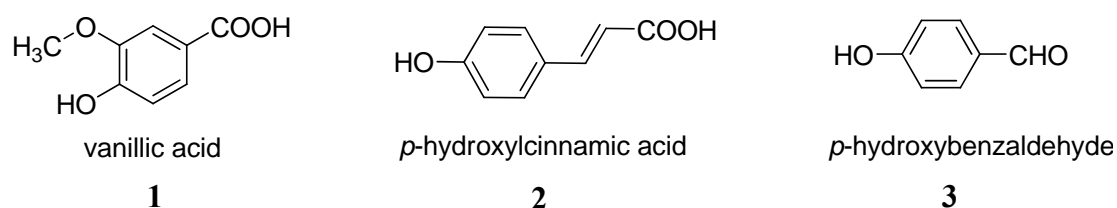
Fig. 1. Chemical structures of compounds **1**-**3** from the root of *S. stoloniferum*.

itive FAB-MS m/z 123 $[M+H]^+$. This compound was identified as *p*-hydroxybenzaldehyde. The structures of the 3 compounds isolated from *S. stoloniferum* are shown in Fig. 1.

Determination of α-glucosidase inhibition activity

The inhibitory effect of the fractions and the isolated compounds against α-glucosidase type IV from *S. stearothermophilus* were evaluated and the results are presented in Table 1. The SS-M-EA, SS-M-C and SS-M-H possessed 72.71%, 42.76% and 26.49% inhibition activity at the concentration of 10 μg/mL, respectively. However, SS-M-B and SS-M-W displayed weak activity against α-glucosidase. Compounds **1** and **3** indicated 20.13% and 30.42% inhibition activity at the concen-

tration of 10 μg/mL, respectively. However, compound **2** show no α-glucosidase inhibition activity (Table 1).

DPPH radical scavenging activity

The SS-M-EA fraction and the isolated compounds showed a dose-dependent radical scavenging activity by converting the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine derivative. L-Ascorbic acid, a known antioxidant was used as a positive control. The SS-M-EA showed a potent DPPH free radical scavenging activity, with an IC_{50} value of 24.37 ± 0.88 μg/mL (Table 2). The DPPH radical scavenging activity of the isolated compounds decreased as follows: *p*-hydroxylcinnamic acid > vanillic acid > *p*-hydroxybenzaldehyde.

DISCUSSION

Diabetes mellitus (DM) has become a major public health problem worldwide. Two hundred and twenty million individuals are estimated to have diabetes in 2010 and 300 million in 2025. Type-2 diabetes (non-insulin-dependent diabetes mellitus) accounts for more than ninety percent of all cases of diabetes (11). Traditional medicinal plants and microorganisms are good sources of α-glucosidases inhibitors, such as acarbose, 1-deoxynojirimycin, and genistein (12), which help fight against diabetes. Some synthetic α-glucosidases inhibitors have undesirable side effects, such as abdominal cramping and diarrhea, and some inhibitors may increase the chance of hepatic syndrome and renal tumors (13, 14). Additionally, glycosidase inhibition has also become an important therapeutic target for cancer and viral infections, including HIV and influenza, with a number

Table 1. Inhibitory effect of fractions and isolated compounds of *S. stoloniferum* against α-glucosidase

Sample	Conc. of sample (μg/mL)	α-glucosidase inhibition (%)
SS-M-H	10	26.49 ± 0.78
	50	50.61 ± 1.03
	100	65.59 ± 0.59
SS-M-C	10	42.77 ± 0.92
	50	67.23 ± 0.38
	100	89.93 ± 0.87
SS-M-EA	0.1	54.51 ± 0.95
	1	59.51 ± 1.37
	10	72.71 ± 1.44
SS-M-B	50	14.90 ± 0.89
	100	22.67 ± 0.79
	500	25.14 ± 1.86
SS-M-W	100	12.75 ± 2.64
	500	30.28 ± 0.95
	1000	38.63 ± 1.63
Vanillic acid	10	20.13 ± 0.97
	50	30.06 ± 1.57
	500	34.78 ± 1.49
p-hydroxybenzaldehyde	10	30.42 ± 0.67
	100	40.63 ± 1.05
	500	43.75 ± 1.76
Acarbose	0.01	48.08 ± 0.93

SS-M-H, SS-M-C, SS-M-EA, SS-M-B, and SS-M-W represent *n*-hexane, CHCl₃, EtOAc, *n*-BuOH, and water fraction from methanol extract of *S. stoloniferum* respectively. Values are the mean ± SD of three replicates.

Table 2. DPPH radical scavenging activities of fractions and isolated compounds of *S. stoloniferum*

Sample	IC_{50}
SS-M-EA	24.37 ± 0.88 μg/mL
Vanillic acid	2.10 ± 0.11 μM
p-hydroxylcinnamic acid	1.59 ± 0.08 μM
p-hydroxybenzaldehyde	2.72 ± 0.17 μM
L-Ascorbic acid	12.25 ± 0.39 μg/mL

SS-M-EA represents EtOAc fraction from methanol extract of *S. stoloniferum*. L-Ascorbic acid is the positive control.

of such drugs in current clinical use (15).

Vanillic acid and *p*-hydroxybenzaldehyde are major phenolic compounds with antioxidant activity in vanilla (16). Huang et al. (17) found that vanillic acid might contribute to the prevention of the development of diabetic neuropathy by blocking the methylglyoxal-mediated intracellular glycation system. In the present study, our results revealed that vanillic acid and *p*-hydroxybenzaldehyde exhibited the α-glucosidase inhibition activity, which might be useful for development of new therapies in the field of diabetes, cancer, and viral infections.

Oxidative stress has been shown to be a hallmark of many diseases that involve metabolic or vascular disorders. In view of the typical characteristics of diabetes, oxidative stress has a double impact on both metabolic and vascular functions (18). It was reported that oxidative stress in pancreatic β-cells was caused by hyperglycemia (19). Voglibose, a known α-glucosidase inhibitor, suppressed oxidative stress in pancreas β-cell in rats of type 2 diabetes (20), while N-acetyl-L-cysteine, an antioxidant, plus vitamin C or E complexes protects pancreatic β-cells against glucose toxicity (21). These results suggest that antioxidants might be useful for treating type 2 diabetes. Antioxidant activity was shown by some of the extracts from *S. stoloniferum*.

In conclusion, vanillic acid, and *p*-hydroxybenzaldehyde which were isolated from *Sparganium stoloniferum* possessed potent activity against α-glucosidase, and also indicated they had antioxidant capability. However, the mechanism of the effect of these compounds on diabetes remains unknown and further study is needed.

ACKNOWLEDGMENTS

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