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α-Glucosidase and α-amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (*Bergenia ciliata*, Haw.)

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Abstract

Herbal medicine has been used for many years by different cultures around the world for the treatment of diabetes. The Nepalese herb Pakhanbhed, is one of the traditional remedies used for diabetes since prehistoric times. In this study, we examined the anti-diabetic activity using an *in vitro* model and isolated the active compounds from Pakhanbhed. Extraction and fractionation of the extract lead to the isolation of two active compounds, (−)-3-*O*-galloylepicatechin and (−)-3-*O*-galloylcatechin and these are reported from this plant species for the first time. These isolated compounds demonstrated significant dose dependent enzyme inhibitory activities against rat intestinal α-glucosidase and porcine pancreatic α-amylase. IC_{50} value for sucrose, maltase and α-amylase were 560, 334 and 739 μM, respectively for [(−)-3-*O*-galloylepicatechin] and 297, 150 and 401 μM, respectively for [(−)-3-*O*-galloylcatechin]. Our study, for the first time, revealed the anti-diabetic potential of Pakhanbhed and this study could be helpful to develop medicinal preparations or nutraceutical and functional foods for diabetes and related symptoms.

Keywords: *Bergenia ciliata*; Medicinal herb; α-Glucosidase/α-amylase inhibitor; Diabetes; Nepal

1. Introduction

Diabetes mellitus (DM) is a chronic disease caused by inherited or acquired deficiency in insulin secretion and by decreased responsiveness of the organs to secreted insulin. Such a deficiency results in increased blood glucose level, which in turn can damage many of the body's systems, including blood vessels and nerves (Matsui et al., 2007). DM is currently one of the most costly and burdensome chronic diseases and is a condition that is increasing in epidemic proportions throughout the world (King, Aubert, & Herman, 1998). Diabetes affects about 5% of the global population (WHO, 2002) and the management of diabetes without any side effects is still a challenge to the medical system (Chakraborty & Rajagopalan, 2002; Kameswararao, Kesavulu, & Apparao, 2003).

One therapeutic approach for treating diabetes is to decrease the post-prandial hyperglycemia. This is done by retarding the absorption of glucose through the inhibition of the carbohydrate-hydrolysing enzymes α-glucosidase and α-amylase in the digestive tract. Inhibitors of these enzyme delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the post-prandial plasma glucose rise (Rhabasa-Lhoret & Chiasson, 2004). Many natural resources have been investigated with respect to the suppression of glucose production from carbohydrates in the gut or glucose absorption from the intestine (Fernando, Wickramasingle, Thabrew, Ariyananda, & Karunanayake, 1991; Welsh, Lachance, & Wasserman, 1989).

In many developing countries like Nepal, traditional medicine, in particular herbal medicine is sometimes the only affordable source for healthcare (Bhattarai, 1993; Manandhar, 1995; Shrestha & Joshi, 1993). As for the

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developed countries, the use of herbal medicine by the suffers of chronic disease is encouraged because there is concern about the adverse effects of chemical drugs and treatment using medicines of natural origin appears to offer more gentle means of managing such disease (Hamdan & Afifi, 2004; Klepser & Klepser, 1999; WHO, 2002). Herbal drugs are prescribed widely because of their effectiveness, fewer side effects and relatively low cost. To this end, research has begun to embrace traditional medicines from various cultures, as scientists search for clues to discover new therapeutic drugs for diabetes (Li, Zheng, Bukuru, & De Kimpe, 2004). Traditional Indian and Chinese medicines have long used plant and herbal extracts as anti-diabetic agents (Chen, Feng, Guo, Sun, & Jiang, 2001; Grover, Yadav, & Vats, 2002). Therefore, investigation on such agents from traditional medicinal plants has become more important and researches are competing to find the new effective and safe therapeutic agent for the treatment of diabetes.

In this study we have selected *Bergenia ciliata* from Nepal, to evaluate its anti-diabetic activity. *B. ciliata* is locally known as "Pakhanbhed" in the Nepali language, and is used in traditional ayurvedic medicine for the treatment of several diseases. *B. ciliata* is a herbal medicine commonly used in the treatment of diabetes in rural communities of Nepal, either alone or in combination with other forms of treatment (Ghimire, 2000). *B. ciliata* has been studied for its biological activity. Sinha et al. (2001a) and Mazhar, Azhar, Mazhar, and Usmanghani (2002) have reported the anti-bacterial activity of a *B. ciliata*. Sinha, Murugesan, Pal, and Saha (2001) studied the anti-tussive activity of a *B. ciliata* Sternb. rhizome extract in mice. Similarly, Sinha et al. (2001b) reported the anti-inflammatory potential of a *B. ciliata* Sternb. rhizome extract in rats. Shrestha and Joshi (1993), Manandhar (1995), IUCN (2004) and Rajbhandari et al. (1995) reported the use of *B. ciliata* in a local traditional ayurvedic medicine system in an indigenous community in Nepal. Although the medicinal herb *B. ciliata* is being widely used in the local traditional medicinal treatment system for a variety of diseases in Nepal, India, Pakistan, Bhutan and some other countries, this potential herb has remained uninvestigated for its possible anti-diabetic activity. Hence, the objective of this study was: firstly to evaluate the anti-diabetic activity and secondly to isolate and identify the bioactive compounds of *B. ciliata*. The work is part of a larger investigation aimed at gaining insight into Nepalese traditional medicines and diabetes.

2. Materials and methods

2.1. Materials

The studied herb, *B. ciliata*, (rhizome) was purchased from a local herbal shop in Kathmandu, Nepal. The plants was botanically identified at the National Herbarium Center, Kathmandu. The rat intestinal acetone powder and the porcine pancreatic α-amylase was supplied by Sigma Aldrich Japan Co. (Tokyo, Japan). ICN Alumina B, Akt.-I was purchased from ICN Biomedicals GmbH (Eschwege, Germany). All the chemicals used were of analytical grade and were purchased from Wako Pure Chem. Co. (Osaka, Japan), unless otherwise stated.

2.2. Preparation of crude extracts

The dried plant samples were extracted with 50% aqueous methanol for 24 h at room temperature. The crude extract was obtained by filtration and the extract was evaporated to dryness with a rotary evaporator, under reduced pressure at 40 °C. The dried residue was redissolved in 50% dimethyl sulfoxide (DMSO) and subjected to porcine pancreatic α-amylase (PPA), sucrase, and maltase inhibitory activity assays.

2.3. Assay for rat intestinal sucrase inhibitory activity

The rat intestinal sucrase inhibitory activity was determined using a literature method (Nishioka, Kawabata, & Aoyama, 1998) with a slight modification. Exactly 0.2 ml of 56 mM sucrose in 0.1 M potassium phosphate buffer (pH 7, 0.2 mL) was mixed with 0.1 mL of the plant extracts in 50% aqueous dimethyl sulfoxide (DMSO). After pre-incubation at 37 °C for 5 min, 0.2 mL of rat intestinal α-glucosidase solution prepared from rat intestinal acetone powder was added. Whereas 0.1 mL DMSO was used in place of the plant extract for the blank sample. After thoroughly mixing, both sample and blank test tubes were incubated at 37 °C for 15 min and then the reaction was stopped by adding 0.75 mL of 2 M Tris–HCl buffer (pH 6.9). The reaction mixture was passed through a basic alumina column (ϕ 6 mm × 35 mm h) to eliminate phenolic or acidic compounds. The amount of liberated glucose was determined by the glucose oxidase method using a commercial test kit (Glucose-B Test Kit, Wako Pure Chem. Co., Osaka, Japan). The mixture of 50 μL filtrate and 200 μL glucose kit solution was incubated in a 96-wells microplate at 37 °C for 30 min. The optical density (OD) of the wells was measured at 490 nm and the inhibitory activity was calculated using following formula:

$$\text{Inhibitory activity } (\%) = (\text{OD}_{\text{control}} - \text{OD}_{\text{test sample}})/\text{OD}_{\text{control}} \times 100$$

2.4. Assay for rat intestinal maltase inhibitory activity

Rat intestinal maltase inhibitory activity was determined using a literature method (Toda, Kawabata, & Kasai, 2000) with a slight modification. The assay was carried out in the same manner as the assay for rat intestinal sucrase inhibitory activity (Section 2.3), except for using 3.5 mM maltose in 0.1 M potassium phosphate buffer

(pH 7, 0.35 mL) as a substrate and 0.05 mL rat intestinal α-glucosidase solution was added.

2.5. Porcine pancreatic α-amylase (PPA) inhibitory activity assay

PPA inhibitory activity was determined using a literature method (Hansawasdi, Kawabata, & Kasai, 2000) with a slight modification. Starch azure (2 mg), which was used as a substrate, was suspended in 0.5 M Tris–HCl buffer (pH 6.9) containing 0.01 M $CaCl_2$ and soaked in boiling water for 5 min. Then, the starch azure solution was pre-incubated at 37 °C for 5 min. The test samples (0.2 mL) in 50% DMSO and 0.1 mL of PPA solution (2.189 U/mL, α-amylase from Porcine Pancreases, EC-3.2.1.1, Sigma Chemicals Co.) were added into each assay sample. Whereas 0.1 mL 0.5 M Tris–HCl buffer was used in place of the plant extract for the blank sample. After thoroughly mixing, both the sample and the blank test tubes were incubated at 37 °C for 10 min and the reaction stopped by adding 0.1 mL of 50% acetic acid. The reaction mixture was then centrifuged (3000 rpm, 4 °C) for 5 min. The absorbance of the supernatant, at 595 nm, was measured and the inhibitory activity was calculated using following formula:

$$\text{Inhibitory activity } (\%) = (OD_{control} - OD_{test\ sample})/OD_{control} \times 100$$

2.6. Spectrometric analysis

^{1}H NMR spectra were recorded with an AMX500 instrument (^{1}H, 500 MHz). The sample was dissolved in methanol-d_4. FAB-mass spectra were obtained on a JEOL AX500 spectrometer (JEOL, Tokyo, Japan).

3. Results

Diabetes mellitus is one of the most serious, chronic diseases that is developing along with an increase in both obesity and ageing in the general population. One of the therapeutic approaches for decreasing post-prandial hyperglycemia is to retard absorption of glucose by the inhibition of carbohydrate hydrolyzing enzymes, for example α-amylase and α-glucosidase, in the digestive organs. We therefore investigated the inhibitory effects of a methanol extract from *B. ciliata* on α-glucosidase and α-amylase. Results of this study are briefly discussed in the following sections.

3.1. Screening of plant extracts on α-glucosidase and α-amylase inhibition assays

The crude methanolic extract of *B. ciliata* showed strong inhibitory activity for both α-glucosidase and α-amylase, whereas, after partitioning between water and ethyl acetate, the ethyl acetate soluble fraction demonstrated comparatively higher enzyme inhibitory activities (Table 1).

Table 1
α-Glucosidase and α-amylase inhibition activity of *Bergenia ciliata* extracts

Plant extract	Inhibition (%)[a]		
	α-Glucosidase		α-Amylase
	Sucrase	Maltase	
50% Methanolic crude extract	30.2 ± 4.1	68.6 ± 1.4	93.5 ± 2.1
Water soluble extract	6.8 ± 0.3	19.0 ± 1.1	65.3 ± 2.7
Ethyl acetate soluble extract	6.9 ± 0.7	53.3 ± 1.9	84.3 ± 13.2

[a] Percentage of inhibition was calculated as: inhibitory activity (%) = $(OD_{control} - OD_{test\ sample})/OD_{control} \times 100$; and values are the means ± SEM; $n = 3$.

Due to it having the most inhibitory effect, the ethyl acetate fraction of *B. ciliata* was selected for further investigation, involving bioassay guided fractionation, in order to isolate the constituents responsible for the effect of the plant.

3.2. Isolationand identification of active compounds from B. ciliata

B. ciliata (150 g) was extracted with 50% aqueous MeOH (1000 mL) at room temperature for 24 h. The crude extract was obtained by filtration through filter paper (Whatman No. 5C, 110 mm) and was evaporated and partitioned between EtOAc and H_2O. The active EtOAc-soluble layer was chromatographed on Cosmosil 75C18-OPN (Nacalai Tesque, Inc., Kyoto, Japan) with H_2O–MeOH gradient elution. The active fraction eluted with H_2O–MeOH (70:30) was subjected to HPLC [Column: Inertsil PREP-ODS, 20 × 250 mm; mobile phase: H_2O–MeOH (65:35); flow rate: 5.0 mL/min; detection: UV 254 nm] to yield two active compounds, [**1**] (52.21 mg) and [**2**] (75.58 mg). Spectroscopic data for the compounds [**1**] and [**2**] were: FABMS: *m/z* 441 ($[M–H]^-$); ^{1}H NMR (methanol-d_4) δ (ppm) (J in Hz): 2.84 (1H, dd, $J = 17.5$, 2.0, H-4_{ax}), 2.98 (1H, dd, $J = 17.5$, 4.7, H-4_{eq}), 5.02 (1H, br s, H-2), 5.51 (1H, br s, H-3), 5.95 (2H, br s, H-6 and 8), 6.68 (1H, d, $J = 8.4$, H-5′), 6.79 (1H, dd, $J = 8.4$, 2.0, H-6′), 6.92 (1H, d, $J = 2.0$, H-2′), 6.94 (2H, s, H-2″ and 6″), and FABMS: *m/z* 441 ($[M–H]^-$), ^{1}H NMR (methanol-d_4) δ (ppm) (J in Hz): 2.70 (1H, dd, $J = 16.5$, 6.2, H-4_{ax}), 2.80 (1H, dd, $J = 16.5$ 5.2, H-4_{eq}), 5.05 (1H, d, $J = 5.9$, H-2), 5.36 (1H, m, H-3), 5.93, 5.95 (each 1H, d, $J = 2.5$, H-6 or 8), 6.71 (2H, m, H-5′ and 6′), 6.82 (1H, s, H-2′), 6.95 (2H, s, H-2″ and 6″), respectively. After a comparison of these spectral data (MS, ^{1}H NMR) with the literature database (Barca et al., 2003; Wan, Chen, Dou, & Chan, 2004), these compounds were identified as: (−)-3-*O*-galloylepicatechin [**1**] and (−)-3-O-galloylcatechin [**2**], (Fig. 1).

3.3. Biological activity of isolated compounds

The isolated compounds were further examined for their α-glucosidase and α-amylase inhibitory activities by evaluating their IC_{50} values. The IC_{50} value is the half maximal

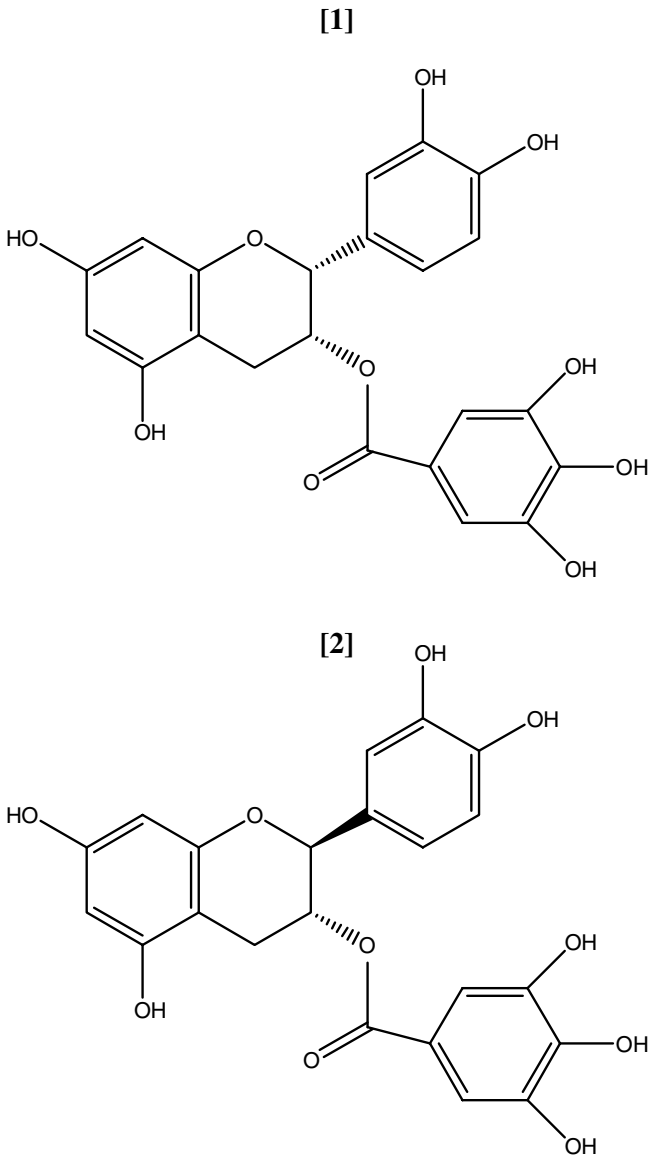

Fig. 1. Structure of compounds isolated from *Bergenia ciliata*: **[1]** (−)-3-*O*-galloylepicatechin, [**2**] (−)-3-*O*-galloylcatechin.

inhibitory concentration, representing the concentration of an inhibitor that is required for 50% inhibition of its targeted enzyme and it is commonly used as a measure of inhibitor effectiveness. Table 2 shows the IC_{50}value of isolated compounds. Although the inhibitory potency was still lower than that of the therapeutic durg, acarbose, observed data clearly indicated the potential of these compounds as inhibitors for α-glucosidase and α-amylase. The IC_{50} values of these two compounds revealed that compound (−)-3-O-galloylcatechin appeared to be a stronger enzyme inhibitor compared with (−)-3-*O*-galloylepicatechin. In general the inhibitory profile demonstrated that the inhibitory activity of isolated compounds was greater against maltase activity compared with sucrase activity. However both of these isolated compounds exhibited dose-dependent inhibitory activities.

Table 2
IC_{50} values of identified compounds from *Bergenia ciliata*

Isolated compounds	IC_{50} values (μM)		
	Sucrase	Maltase	α-Amylase
(−)-3-*O*-galloylepicatechin	560	334	739
(−)-3-*O*-galloylcatechin	297	150	401

4. Discussion

The treatment goal of diabetes patients is to maintain near normal levels of glycemic control, in both the fasting and post-prandial states. Many natural resources have been investigated with respect to suppression of glucose production from carbohydrates in the gut or glucose absorption from the intestine (Matsui et al., 2007). α-Amylase catalyses the hydrolysis of α-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 (Hara & Honda, 1990). Therefore, effective and nontoxic inhibitors of α-amylase and α-glucosidase have long been sought.

In this study we have investigated the anti-diabetic potential of the Nepalese herb *B. ciliata*, which is used in traditional ayurvedic medicine for the treatment of several diseases. This valuable herb was not previously investigated for its anti-diabetic activity. However, our study clearly established the anti-diabetic potential of *B. ciliata* and revealed that the active principles responsible for this activity are (−)-3-*O*-galloylepicatechin and (−)-3-O-galloylcatechin, and these were reported from this plant species for the first time. These isolated compounds were abundantly found in tea. Flavonoids, like anti-oxidants, may prevent the progressive impairment of pancreatic beta-cell function due to oxidative stress and may thus reduce the occurrence of type 2 diabetes (Song, Manson, Buring, Sesso, & Liu, 2005). In their study Kao, Chang, Lee, and Cheng (2006) have reported that these tea catechines, especially (−)-epigallocatechine gallate (EGCG) appears to have anti-obesity and anti-diabetic effects and they have suggested that tea- and EGCG-based folk medicine may be utilised in the treatment of obesity, diabetes and other chronic disease including cancer. Similarly, Sabu, Smitha, and Kuttan (2002) have also reported the anti-diabetic and free radical scavenging activity of tea polyphenols such as gallocatechin (GC), epigallocatechin (EGC), epicatechin (EC), epigallocatechin gallate (EGCG) and epicatechin gallate (ECG). We hypothesise that a higher intake of catechin-rich food by diabetic patients may provide some protection against the development of the long-term complications of diabetes (Rizvi, Anis, Zaid, & Mishra, 2005).

Although, in the present study, the enzyme inhibitory activity of these isolated compounds were assayed *in vitro*, the results from this work should be relevant to the human body. The α-glucosidase and α-amylase inhibitory activity of these isolated compounds have also been reported by using different *in vivo* and *in vitro* models (Barca et al., 2003; Hara & Honda, 1990; He, Lv, & Yao, 2006; Matsui et al., 2007; Wan et al., 2004). In addition to α-amylase and α-glucosidase inhibitory activities, these isolated compounds are also reported to have several other biological activities including anti-bacterial, anti-oxidative, anti-cancer etc. (He et al., 2006). This supportive evidence further increases the medicinal importance of this Nepalese herb, *B. ciliata* indicating that this herb is not only beneficial for diabetes but also may be useful to a number of other human health complications.

5. Conclusion

This study investigated the potential anti-diabetic activity of the Nepalese herb Pakhanbhed, focusing on the inhibitory effects on α-glucosidase and α-amylase. The potential enzyme inhibitor compounds of this herb are identified as (−)-3-*O*-galloylepicatechin and (−)-3-O-galloylcatechin and were reported from this plant species for the first time. Our study is the first to report a potential mode of action of *B. ciliata* and suggests that the glucose lowering effect of this plant is due to the inhibition of digestive enzymes, α-glucosidase and α-amylase. In conclusion, the results from this study give scientific support to the use of *B. ciliata* in folklore medicine for the treatment of diabetes and show, for the first time, the potential role of α-glucosidase and α-amylase inhibition in its activity. This study would be helpful to explain the pharmacological mechanism and also to develop medicinal preparations, nutraceuticasl or functional foods for diabetes and related symptoms.

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