

α -Glucosidase and α -Amylase Inhibition Activities of *Sarcococca coriacea* Hook. And *Sarcococca wallichii* Staph. of Nepalese Origin

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Submitted : 17 June 2022, Revised 28 June 2022, Accepted 29 June 2022

Abstract

Diabetes mellitus is being severe health problem globally with increasing patients every day. Due to lack of effective and non-toxic medicine to cure diabetes. Plants that are used in ethnomedicine may be a good source for antidiabetic drug discovery. Plants of the *Sarcococca* genus are medicinally important and are used by local people for managing many diseases including diabetes. In the course of our continuous search of antidiabetic plants and pure compounds, *in vitro* α -glucosidase, and α -amylase inhibition activity along with the antioxidant activity of methanolic extract of *Sarcococca coricea* leaf (Sc-A), *Sarcococca coricea* stem (Sc-B), and dichloromethane fraction of methanolic extract of *Sarcococca wallichii* (Sw-D) were carried out. The research revealed dicholoromethane fraction of *S. wallichii* (Sw-D) with good inhibition of α - amylase enzyme ($IC_{50} = 53.79 \pm 2.50$), whereas Sc-B inhibits α -glucosidase (20.97 ± 2.37) effectively. Similarly, Sc-A showed significant antioxidant activity with $IC_{50} = 24.56 \pm 3.3$. The total phenolic content on Sc-A and Sc-B was calculated as 151.35 ± 4.42 mg GAE/g and 86.22 ± 1.59 mg GAE/g whereas the total flavonoid content on Sc-A and Sc-B was found to be 21.61 ± 4.88 mg QE/g and 24.09 ± 4.02 mg QE/g respectively. Similarly, total phenolic and total flavonoid content on Sw-D were found to be 85.26 ± 3.16 mg GAE/g and 21.57 ± 1.26 mg QE/g. To the best of our knowledge, this is the first report of α -glucosidase and α -amylase inhibition activity in these plants. This research work has scientifically supported the use of these plants to manage diabetes by local people and has explored new plants for antidiabetic drug discovery research.

Keywords: α -glucosidase inhibitor, α -amylase inhibitor, antioxidant, *Sarcococca*

Introduction

Diabetes mellitus, commonly called type 2 diabetes identified by hyperglycemia is caused due to various degrees of β cell dysfunction and insulin resistance. Patients with diabetes mellitus are increasing globally with an associated two-fold excess risk of cardiovascular, cerebrovascular, and peripheral artery disease [1], [2]. The search for potent antidiabetic secretagogue from plant origin is in high demand due to its low economic cost and lesser side effect. As the consumed food has a direct impact on the blood glucose level. The enzymatic digestion of complex

carbohydrates collectively by the role of α -amylase and α -glucosidase has been addressed as a potential means of controlling postprandial hyperglycemia by reducing the absorption of glucose from meals [3]. Inhibitors of these enzymes help by delaying in breaking mechanism of carbohydrates finally leading to a decrease in the postprandial glucose level in the blood [4]. Various researches targeting the plant-based inhibitors are on an increasing trend against these digestive enzymes α -amylase and α -glucosidase as natural supplement and diet plays a pivotal role in addressing the concern related to

synthetic drugs. Initiation of such research through *in vitro* antidiabetic property of plant extract is expressed by the inhibition of plant sample to the enzymes α -amylase and α -glucosidase[5]. Plants with the highest inhibitory activities against both of these digestive enzymes are rare. Very few natural plants are reported with a strong inhibiting capacity to both these enzymes concomitantly[6]. Plants belonging to Buxaceae family are reported to have potent to moderate inhibitory capacity in multiple diseases and need to be researched concerning antidiabetic properties.

The evergreen Buxaceae family have four species of *Sarcococca* reported from the various belt of Nepal, *S. coriacea*, *S. saligna*, *S. hookerina* & *S. wallichii*[7]. These plants are rich in steroid alkaloids. The nature of compound in plants are responsible for the impact of the result observed. The value of consumption of natural plant products as herbal remedies in treating certain ailments and disorders is a common practice in Nepal. Locally people use the bark of *Sarcococca coriacea* to get relief from swelling[8]. Similarly, leaves and shoots of *Sarcococca* plants have been used for the treatment of rheumatic fever in folk medicine[9]. Whole parts of this medicinally important plant roots, stem, leaves, bark, and flowers have been continuously under multidimensional intense research for years.

The importance of these genera is reflected in many types of research with biological activities ascribed to isolated steroid alkaloids. The alkaloids are pregnane derivatives [10]. Potent biological activities include acetylcholinesterase and butyrylcholinesterase inhibition in various concentrations of compounds of class pregnane type steroid alkaloids namely hookerianamide-D, hookerianamide-E, hookerianamide F and hookerianamide G isolated from *Sarcococca hookeriana* [11] in addition to the antileishmanial property [12] Compounds of *Sarcococca hookeriana* as hookerianamide H, hookerianamide I, N-methylepipachysamine D, Sarcovagine C, and dictyophlebine possess both cholinesterase and antiplasmoidal [13] properties with good inhibitory activities. Leaves of *Sarcococca coriacea* of Nepalese origin contains potent compounds

such as (-) vaganine D, (+) nepapakistamine A with cholinesterase inhibitory activities. Likewise other steroid alkaloids isolated from leaves were epoxypakistamine-A, epoxysarcovageneine D, Funtumafrine C, and N-methylfuntumine [14]. All these compounds except Funtumafrine C possess potent acetylcholinesterase and butyrylcholinesterase inhibition activity in a concentration-dependent pattern. Breakthrough in research was observed when flourine containing secondary metabolite has been reported from a natural source for the first time from *Sarcococca coriacea*, a novel class of fluoropyrimidine substituted alkaloids along with many steroid alkaloids with potent biological activities [15]. Others isolated compounds were alkaloid C, Na-methylepipachysamine D, Sarcovageneine, Sarcovagine D, N-methylepipachysamine A, dictyophlebine, 5,6-dihydrosarconidine, terminaline and iso-N formylchoenmorphine. Roots of *Sarcococca coriacea* possess anti-leishmanial activities which are also observed due to steroid alkaloids such as iso-N-formylchonemorphine and alkaloid C [16]. Rare sugar alcohol, xylitol along with other sterols were reported from flowers of *Sarcococca coriacea* [17]. These all researches strongly add the medicinal value of the plant under this genera. Owing to the strong potency of all these species from Buxaceae family this investigation continues to the *in-vitro* application of antidiabetic property of *Sarcococca coriacea* leaf, *Sarcococca coriacea* stem and *Sarcococca wallichii* dichloromethane fractions as to the best of our knowledge the antidiabetic potential of these plants have not been reported till the date.

Materials and Methods

Plant Collection and Extract Preparation

Sarcococca coriacea plant was collected from Kathmandu district, Kritipur Municipality, Champadevi-4 [27.66 °N, 85.27 °E, 2229 m, 16 July, 2018, J. Baral & Y.B. Poudel, JB100 (KATH)]. The leaves and stems of the plants were separated air-dried and ground to powder. With occasional shaking, both leaves and stem powder were macerated in methanol for ten days. It was then filtered, and the filtrate was concentrated to obtain the crude extract in a rotary evaporator. Similarly, this section of

research on *Sarcococca wallichii* is in continuation of our previous research [18] where the aerial part of *Sarcococca wallichii* Staph. were collected from Dhampus (Kaski, Nepal) and identified by Prof. Krishna Kumar Shrestha, Central Department of Botany, Tribhuvan University, Kritipur, Kathmandu, Nepal. A voucher specimen (No: SW-06) was deposited in the same department.

Chemicals

2,2 Diphenyl 1 picrylhydrazyl (DPPH), α -glucosidase from *Saccharomyces cerevisiae*, α -amylase from porcine pancreases, 2-Chloro-4-nitrophenyl- α -D-Maltotrioside (CNPG3) and *p*-Nitrophenyl- α -D-glucopyranoside (PNPG) were purchased from Sigma-Aldrich.

Total Phenolic Content (TPC)

Folin-ciocalteu's reagent was used for the estimation of total phenolic content [19]. In short, the plant samples (20 μ L) were mixed with Folin-Ciocalteu's reagent (100 μ L) and Na₂CO₃ (80 μ L) and left in the dark for 15 minutes. Finally, absorbance was measured at 765nm in a microplate spectrophotometer

Total Flavonoid Content (TFC)

The aluminium trichloride method was used to estimate the total flavonoid content [20]. Briefly, plant sample (20 μ L), distilled water (60 μ L), AlCl₃ (5 μ L), and CH₃Cook (5 μ L) were mixed and kept for 30 minutes at room temperature. The absorbance was then measured at 415nm using a microplate spectrophotometer.

Antioxidant Activity

The determination of antioxidant activity was done by following the previously described method with slight modification [21]. The plant sample (100 μ L) was mixed with DPPH (100 μ L) and kept in the dark for 30 minutes. Finally, the absorbance was taken at 517nm in a microplate spectrophotometer. The free radical scavenging activity was calculated using the following formula.

$$\% \text{ inhibition} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100$$

Where A_{control} is the absorbance of the control and A_{sample} is the absorbance of the sample.

In vitro α -Glucosidase Inhibition Activity

The *in vitro* α -glucosidase inhibition assay was performed following the methods described previously with slight modifications[22]. Different concentrations of 20 μ L plant sample were mixed with 20 μ L of the enzyme (0.2U/mL) with 120 μ L of phosphate buffer solution and incubated at 37°C for 15 minutes. After that, PNPG (0.7mM) was added and again incubated for 15 minutes at 37°C. Finally, the absorbance was taken at 405nm in a microplate spectrophotometer. The inhibition percentage was calculated using the following formula.

$$\% \text{ inhibition} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100$$

Where A_{control} is the absorbance of the control (DMSO), and A_{sample} is the absorbance of the sample.

In vitro α -Amylase Inhibition Activity

Substrate-based α -amylase inhibition assay was performed using CNPG3 as substrate[23]Different concentrations of 20 μ L plant sample were first mixed with 80 μ L of the enzyme (1.5U/mL) and incubated for 15 minutes at 37°C. After that, 100 μ L of CNPG3 (0.5mM) was added and incubated for 15 minutes at 37°C. Lastly, the absorbance was taken at 405nm in a microplate spectrophotometer. The inhibition percentage was calculated using the following formula.

$$\% \text{ inhibition} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100$$

Where A_{control} is the absorbance of the control (DMSO), and A_{sample} is the absorbance of the sample.

Results

Total Phenolic and Flavonoid Content

The total phenolic and flavonoid content was expressed as mg GAE/g and mg QE/g, respectively. A calibration curve of gallic acid and quercetin was used to estimate TPC and TFC, respectively. The TPC and TFC of Sc-A were found to be 130.07±5.74 mg GAE/g and 20.58±3.82 mg QE/g, respectively.

Similarly, the TPC and TFC of Sc-B were 68.34 ± 2.57 mg GAE/g and 24.5 ± 6.5 mg QE/g, respectively. The total phenolic and total flavonoid content of Sw-D were found to be 85.26 ± 3.16 mg GAE/g and 21.57 ± 1.26 mg QE/g. The total phenolic content on all three extracts was higher than the corresponding flavonoid content.

Antioxidant Activity

DPPH free radical scavenging assay was performed to determine the antioxidant activity. Both Sc-A and Sc-B were found to significantly inhibit the DPPH free radical with IC_{50} value $24.56 \pm 3.3 \mu\text{g/mL}$ and $28.90 \pm 5.22 \mu\text{g/mL}$, respectively. Comparatively the other species, Sw-D fraction has $IC_{50} = 53.79 \pm 2.50 \mu\text{g/mL}$. Among all three extract Sc-A possess stronger antioxidant potential. The antioxidant potent of standard quercetin was $IC_{50} = 1.17 \pm 0.35 \mu\text{g/mL}$.

In vitro α -glucosidase and α -amylase inhibition activities

For the *in vitro* α -glucosidase and α -amylase inhibition activity, samples were first screened at the concentration of $500 \mu\text{g/mL}$. Samples inhibiting $>50\%$ were further diluted to calculate the IC_{50} value. The IC_{50} was calculated using GraphPad Prism 8 software. The Sc-A showed significant inhibition against both digestive enzymes; α -glucosidase and α -amylase, with an $IC_{50} = 39.92 \pm 2.52 \mu\text{g/mL}$ and $224.3 \pm 1.87 \mu\text{g/mL}$, respectively. Likewise, Sc-B disclosed significant inhibition against α -glucosidase with $IC_{50} = 20.97 \pm 2.37 \mu\text{g/mL}$; however, it showed $<50\%$ inhibition against the α -amylase. Other species Sw-D significantly inhibits α -amylase $IC_{50} = 2.116 \pm 0.058 \mu\text{g/mL}$ than α -glucosidase $< 50\%$ comparatively. Standard Acarbose's IC_{50} against α -glucosidase and α -amylase were found to be $5.66 \pm 0.8 \mu\text{g/mL}$ and $6.18 \pm 0.97 \mu\text{g/mL}$, respectively. This proves the stronger inhibition capacity of Sw-D than standard acarbose used. The IC_{50} of α -glucosidase and α -amylase inhibition activity of Sc-A, Sc-B, and Sw-D compared to the standard acarbose is presented in table 1.

Table 1: IC_{50} values of antioxidant, α -glucosidase and α -amylase inhibition activities of plant's extract and fraction

Plant sample and standards	Antioxidant($\mu\text{g}/\text{mL}$)	α -Glucosidase ($\mu\text{g}/\text{mL}$)	α -Amylase ($\mu\text{g}/\text{mL}$)
Sc-A	24.56 ± 3.3	39.92 ± 2.52	224.3 ± 1.87
Sc-B	28.90 ± 5.22	20.97 ± 2.37	NC
Sw-D	53.79 ± 2.50	NC	2.116 ± 0.058
Quercetin	1.17 ± 0.35	-	-
Acarbose	-	5.66 ± 0.8	6.18 ± 0.97

Values are expressed as average \pm standard deviation of three independent assays. NC-not calculated

Discussions

In vitro potency of plant's extract and fractions provides a platform for further research in the molecular label. Synergetic effect of active phytochemicals such as alkaloids, tannins, phenols, saponins, terpenoids, flavonoids, steroids, and sterols contribute to the plant's medicinal properties[24]. Due to the adverse effect of synthetic drugs the efficacy of plant-based food profiles with potent inhibitors is in demand. Major hydrolyzing enzymes of carbohydrate metabolism are the α -glucosidase and α -amylase. Inhibiting these enzymes may result in controlling blood sugar[25]. This research reveals the unique *in vitro* antidiabetic potency of Sc-A and Sc-B as these both inhibited the digestive enzymes, α -glucosidase, and α -amylase. The methanolic stem extract of the plant *Sarcococca coriacea* inhibited α -glucosidase substantially more than its leaf extract whereas stem extract of the same at the concentration of $500 \mu\text{g/mL}$ could not inhibit the α -amylase enzyme. Leaf extract Sc-A was found to inhibit the α -amylase comparatively. Interestingly another species Sw-D was found to be one of the potent inhibitors of α -amylase that could not inhibit the α -glucosidase enzyme at the concentration of $500 \mu\text{g/mL}$. Sc-A possesses higher phenolic and stronger antioxidant potential. Enzyme inhibition activity of both methanolic extract of Sc-A, Sc-B and Sc-D accredit to the synergetic effect of phytoconstituent present in it.

Conclusions

Sc-A and Sc-B both inhibited digestive enzymes, α -glucosidase, and α -amylase. Sw-D strongly inhibited α -amylase stronger than the standard reflecting remarkable anti-diabetic potential. All three extracts possess good to moderate antioxidant

properties. Our findings infer a strong biochemical rationale for further *in vivo* studies and as a dietary supplement of this plant-based product in type II diabetes management. Further researches are necessary on particular inhibiting compounds and biological pathways involved.

Funding

This research is financially supported by University Grants Commissions (UGC), Nepal to Mrs. Janaki

Baral through Ph. D. Fellowship and Research Support (Award No. PhD/074-075/S&T-6).

Acknowledgments

A. Adhikari would like to acknowledge HERP, Tribhuvan University, Nepal, for providing research support.

Conflict of Interest

The authors declare that there is no conflict of interest.

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