

Inhibition of α -amylase and α -glucosidase activities in vitro by extracts of selected medicinal plants

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Manuscript received: 29 November 2020. Revision accepted: 9 February 2021.

Abstract. Pant DR, Aryal B, Pun D, Sharma S, Joshi GP. 2021. Inhibition of α -amylase and α -glucosidase activities in vitro by extracts of selected medicinal plants. *Biodiversitas* 22: 1187-1193. Several medicinal plants are being used traditionally in the treatment/management of Diabetes Mellitus (DM). The present work focuses on experimental verification of antidiabetic potential of different medicinal plants that have been reported to be used traditionally to manage DM. Aqueous and methanolic extracts of 12 species of plants were studied for their inhibitory effect on the activities of α -amylase and α -glucosidase, two key enzymes of carbohydrate metabolism. The extracts of all the species showed very high degree (nearly 90% or above) of inhibition of α -amylase irrespective of the extraction solvent. However, the aqueous extracts of *Asterella wallichiana*, showed only 63.60% inhibition. Except for *Asterella wallichiana*, the percentage inhibition of α -amylase in aqueous and methanolic solution in all the species tested were almost similar. The percentage inhibition of α -glucosidase was lower than that of α -amylase for all the species in both types of extraction medium. The highest percentage inhibition of α -glucosidase (81.13%) was found in methanolic extracts of *Rhuschinensis*. The inhibition of α -glucosidase was much higher in methanolic extracts than in aqueous extracts for all the species tested. High degree of inhibition of α -amylase activity in vitro by the extracts of all the species tested provides scientific basis for using these plants in the management/treatment of diabetes in traditional medicine.

Keywords: medicinal plants, methanolic extracts, diabetes, α -amylase, α -glucosidase

INTRODUCTION

Diabetes Mellitus (DM) is one of the most important public health problems faced by human society. An estimated 422 million adults were living with diabetes in the year 2014 (WHO 2016). DM is a physiological disorder in which blood sugar level increases and various metabolic activities like carbohydrate, fat, and protein metabolism are disturbed. It is caused either by deficiency in insulin or inability of insulin to control blood glucose level. The former is called type 1 while the latter one is called type 2 diabetes (Farzaei et al. 2017). Among DM patients, about 10% suffer from type 1 DM which normally develops as a result of a chronic progressive autoimmune disorder (Pietropaolo and Roith 2001). However, type 2 DM which accounts for more than 90% of diabetes worldwide, develops with increasing age (CDC 2007). Though, the exact cause of DM is not known for certain, the free radicals are believed to be involved in diabetes and related complications (Baynes and Thorpe 1996; Lipinski 2001).

Plants, the inexpensive natural source of antioxidants, are excellent antidiabetic medicines (Marles and Farnsworth 1995). There are 800 species of plants reported to have antidiabetic activity (Patil et al. 2011). Different groups of plants including bryophytes (Mukhia et al. 2017), pteridophytes (Ibraheim et al. 2011; Paul et al. 2012), and higher plants (Shim et al. 2003; Sah et al. 2015; Pranitha et al. 2014; Manandhar et al. 2011) have been reported to be used in the management/treatment of diabetes. Among the

antidiabetic plants reported so far, the higher plants which account for nearly 95% of total extant flora are the most extensively studied group.

One of the approaches taken in managing DM, especially type 2 diabetes, is the use of various types of oral drugs with different modes of action to control hyperglycemia. The use of α -glucosidase inhibitors like acarbose is one of such approaches (reviewed in Bosenberg and van Zyl 2008). Different medicinal plants used in the management of diabetes may also possess such inhibitors. Therefore, the study of the inhibitory potential of extracts of plants in question against different carbohydrate metabolism enzymes like α -amylase and α -glucosidase can be used as a tool to verify the antidiabetic effects of plant extracts in vitro.

In Nepalese context, studies on antidiabetic potential of medicinal plants are mostly related to documentation of ethnomedicinal aspects of plants in different parts of the country (Singh and Kumar 2017; Dahal 2016; Joshi 2011). There have been very few studies on experimental verification of the potential of those plants for diabetes control (Bhandari et al. 2008; Manandhar et al. 2012; Sah et al. 2015). Therefore, the present work focuses on experimental verification of antidiabetic potential of selected medicinal plants and their allied species belonging to different genera representing various groups like bryophytes (*Asterella*, *Marchantia*), pteridophytes (*Adiantum*, *Oleandra*, and *Tectaria*) and flowering plants (*Hedychium*, *Rhus*, *Rubus*, *Smilax*, and *Sonchus*) in terms

of their ability to inhibit activities of α -amylase and α -glucosidase in vitro.

MATERIALS AND METHODS

Plant materials

Altogether, 12 plant species belonging to ten genera were selected for their antidiabetic potential in vitro. Plant samples of selected species and voucher specimens were collected from different parts of central Nepal (Table 1, Figure 1). Voucher specimens of respective plant materials were used for identification and authentication. The herbaria were deposited at the Tribhuvan University Central Herbarium (TUCH).

Sample preparation

The samples were cleaned and air-dried in shade until constant weight. The dried materials were ground to fine powder using grinder. The powdered mass was sieved through normal sieve and the fine powder was used for extraction procedure.

Extract preparation

Five gram of powder of each sample was taken and divided equally into two falcon tubes. Then respective solvent (water or methanol) was added to each tube up to 50 mL mark. The tubes were closed and vortexed briefly. The tubes were then subjected to sonication at 40 KHz in the Sonicator (E-Chrom Tech, Taiwan UC-7240BDT) for 1 hour and filtered through Whatman No. 1 filter paper. The residue was subjected to solvent extraction as before and then filtered. Both filtrates were mixed and concentrated under reduced pressure in a rotary evaporator (RE 100 PRO, Dragon Lab China). The condensed extract thus obtained was transferred to clean and pre-weighed Petri plate. It was then allowed to evaporate at room temperature under a laminar airflow hood with regular blowing till constant weight. The dried extract was scraped off with a blade and collected in 2 mL polypropylene vials and stored at 4 °C until further use.

Evaluation of antidiabetic activity

Antidiabetic activity of the plant extracts in vitro was assessed by using inhibition assays for the enzymes of carbohydrate metabolism: α -amylase and α -glucosidase. α -amylase inhibition assay was carried out following the protocol adopted by Ahmed et al. (2009) with slight modification. Reaction medium was prepared by dissolving Porcine pancreatic amylase (Sigma Aldrich, Germany) in 0.1M potassium phosphate buffer (pH 6.8) to a final concentration of 0.1 unit/mL. Ten μ L of either pure solvent (Control) or solutions of Acarbose or plant extracts in respective solvents (Samples) were mixed with 390 μ L of reaction medium in two test tubes. In one of the test tubes (Blank), 200 μ L of DNS reagent (Sigma Aldrich, Germany) was added to the above reaction mixture. The tubes were pre-incubated at 37°C for 10 minutes. Then 200 μ L of 1% soluble starch (Fisher Scientific, India) was added and the tubes were incubated at 37 °C for another 20 minutes. Then 200 μ L of DNS reagent was added in all the remaining tubes to stop the reaction. The tubes were then kept in boiling water bath for 10 minutes and then allowed to cool. The resultant mixture was then diluted by adding 4 mL of distilled water in each tube and absorbance was taken at 540 nm (A_{540}) in a UV/Vis spectrophotometer (E-Chrom Tech, Taiwan). Inhibition of α -amylase activity was calculated by using the formula:

$$\alpha\text{-Amylase Inhibition (\% activity)} = 100 * (A_{540}\text{Control} - A_{540}\text{Sample}) / A_{540}\text{Control}$$

Similarly, α -glucosidase inhibition assay was carried out following Si et al. (2010) with slight modification. Reaction mixture was prepared by dissolving p-nitrophenyl α -D-glucopyranose (Sigma Aldrich, Germany) in 0.1M potassium phosphate buffer (pH 6.4) to a final concentration of 10 mM. The reaction mixture was dispensed to 96 well plates at the rate of 150 μ L per well. To the reaction mixture 10 μ L of pure solvent or solutions of Acarbose (5 mg/mL) or plant extracts (1 mg/mL) in respective solvents (water/methanol) were added. Respective blanks were prepared for each of the pure solvents and solution of plant extracts in respective solvents.

Table 1. Details of species, parts used and collection locality of plant samples

Species	Parts used ^{a)}	Collection locality ^{b)}	Elevation (m. asl)	Latitude and longitude
<i>Adiantum incisum</i> Forssk.	WP	I	1200	27° 43' 27" N; 85° 06' 32" E
<i>Asterella wallichiana</i> (Lehm.) Grolle	WP	II	2100	28° 13' 03" N; 83° 47' 34" E
<i>Hedychium coronarium</i> J. Koenig	RH	III	2050	27° 34' 54" N; 85° 23' 20" E
<i>Marchantia polymorpha</i> L.	WP	III	1515	27° 35' 35" N; 85° 22' 55" E
<i>Oleandra wallichii</i> (Hook.) C. Presl	RH	IV	2500	27° 35' 40" N; 85° 04' 58" E
<i>Rhus chinensis</i> Mill.	BK	V	1520	27° 33' 16" N; 85° 37' 34" E
<i>Rhus succedanea</i> L.	BK	V	1520	27° 33' 16" N; 85° 37' 34" E
<i>Rubus niveus</i> Thunb.	LS	VI	1900	27° 38' 28" N; 85° 15' 46" E
<i>Smilax aspera</i> L.	LV	III	1690	27° 37' 16" N; 85° 24' 06" E
<i>Smilax ferox</i> Wall. ex Kunth	LV	III	1680	27° 37' 16" N; 85° 24' 05" E
<i>Sonchus arvensis</i> L.	LV	VI	1320	27° 40' 58" N; 85° 17' 12" E
<i>Tectaria coadunata</i> (Wall. ex Hook. and Grev.) C. Chr.	YF	VII	800	28° 08' 41" N; 84° 24' 42" E

Note: ^aWP: whole plant; RH: rhizome; BK: bark; LS: leafy shoot; LV: leaves; YF: young fronds. ^bI: Dhading, II: Parbat, III: Lalitpur, IV: Makawanpur, V: Kavrepalanchowk, VI: Kathmandu, VII: Lamjung

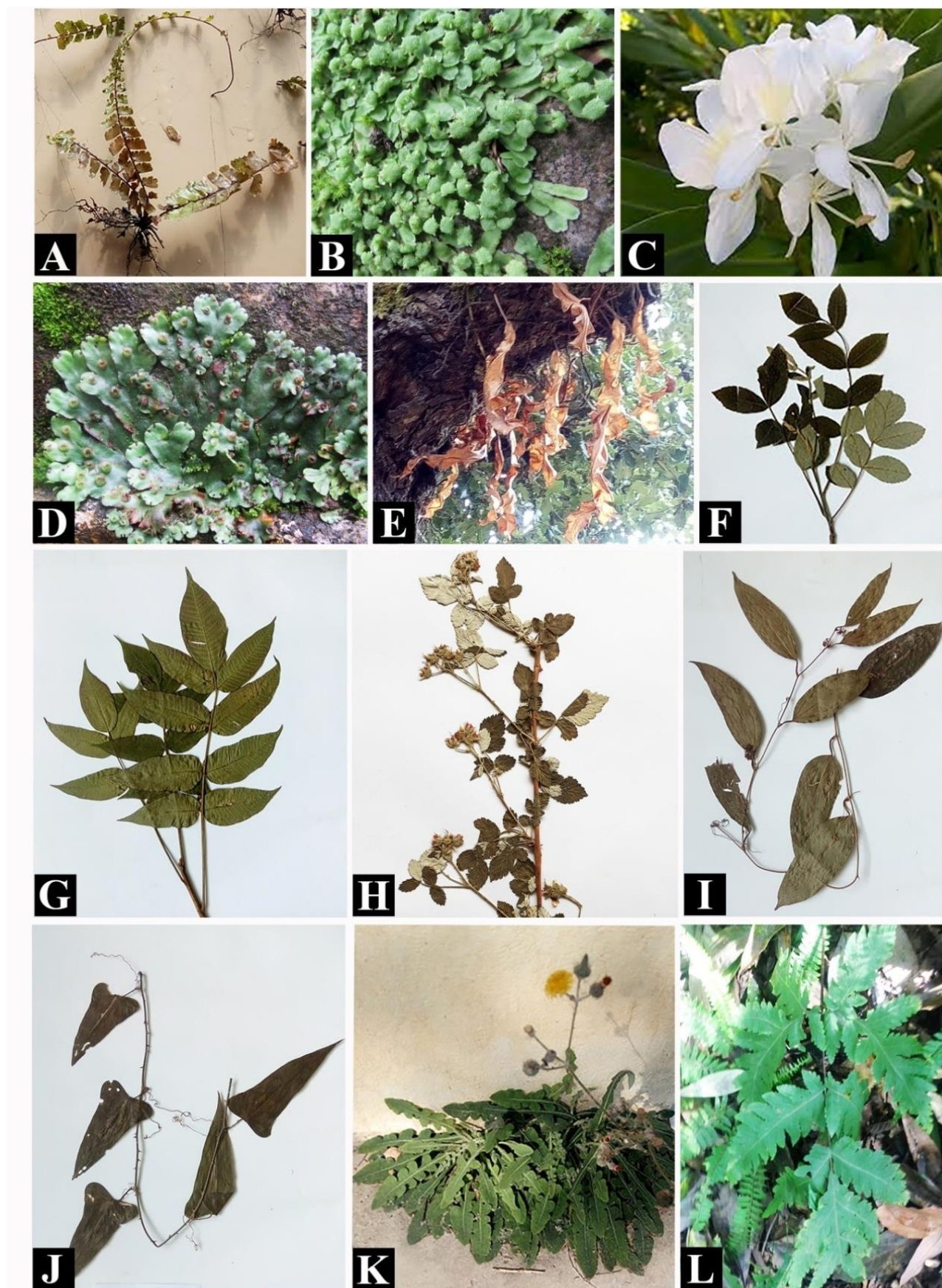


Figure 1. Photographs of selected medicinal plants: A. *Adiantum incisum*, B. *Asterella wallichiana*, C. *Hedychium coronarium*, D. *Marchantia polymorpha*, E. *Oleandra wallichii*, F. *Rhus chinensis*, G. *R. succedanea*, H. *Rubus niveus*, I. *Smilax aspera*, J. *S. ferox*, K. *Sonchus arvensis*, and L. *Tectaria coadunata*

Then 40 μL of 1M Na_2CO_3 was added in all the wells representing the blank. The reaction mixture was pre-incubated at 30 °C for 10 minutes. After 10 minutes, 10 μL of α -glucosidase (Sigma Aldrich, Germany) solution (0.2 U/mL) prepared in phosphate buffer was added and the reaction mixture was incubated at 30 °C for 15 min. Then 40 μL of 1M Na_2CO_3 was added in all the remaining wells. The absorbance was measured at 405 nm (A_{405}) in MultiscanEX (Thermo Fisher) Eliza plate reader. Inhibition of α -glucosidase activity was calculated by using the formula:

$$\alpha\text{-Glucosidase Inhibition (\%)} = 100 * (A_{405} \text{ Control} - A_{405} \text{ Sample}) / A_{405} \text{ Control}$$

Data analysis

All the experiments were performed in triplicates for each sample and values were reported as mean \pm S.D. All calculations were done using Microsoft Excel 2010.

RESULTS AND DISCUSSION

Inhibition of α -amylase activity in vitro by plant extracts

Inhibition of α -amylase activity by extracts of selected antidiabetic plants is shown in Figure 2. Except for aqueous extracts of *Asterella wallichiana*, all other extracts showed inhibition of α -amylase activity comparable to that of acarbose, a standard antidiabetic drug. Among the aqueous

extracts, the highest inhibitory activity (99.15 ± 1.48) and the lowest inhibitory activity (63.6 ± 6.4) was found in extracts of *Smilax ferox* and *Asterella wallichiana*, respectively. In methanolic extracts, the highest percentage inhibition of α -amylase (99.51 ± 0.85) was found in *Smilax aspera* extracts while the lowest percentage inhibition (90.00 ± 6.93) was found in *Oleandra wallichii* extracts.

Inhibition of α -glucosidase activity in vitro

Inhibition of α -glucosidase activity by extracts of selected medicinal plants is shown in Figure 3. In all species, the methanolic extracts showed high degree of inhibition of α -glucosidase activity over aqueous extracts. Among the aqueous extracts, the highest inhibitory activity (21.64 ± 0.7) and the lowest inhibitory activity (5.89 ± 0.41) was found in extracts of *Hedychium coronarium* and *Adiantum incisum*, respectively. The inhibition of α -glucosidase activity in aqueous extracts was comparable to acarbose in *Asterella wallichiana*, *Rhus succedanea*, *Smilax ferox*, and *Tectaria coadunata*, and higher than that of Acarbose in *Marchantia polymorpha*, *Oleandra wallichii*, *Rhus chinensis*, *Rubus niveus* and *Smilax aspera*. In the case of methanolic extracts, the highest percentage inhibition of α -glucosidase (81.13 ± 1.36) was found in *Rhus chinensis* extracts while the lowest percentage inhibition (21.22 ± 1.07) was found in *Smilax aspera* extracts. With the exception of *Smilax aspera* the extracts of all other species showed higher inhibition of α -glucosidase activity than acarbose.

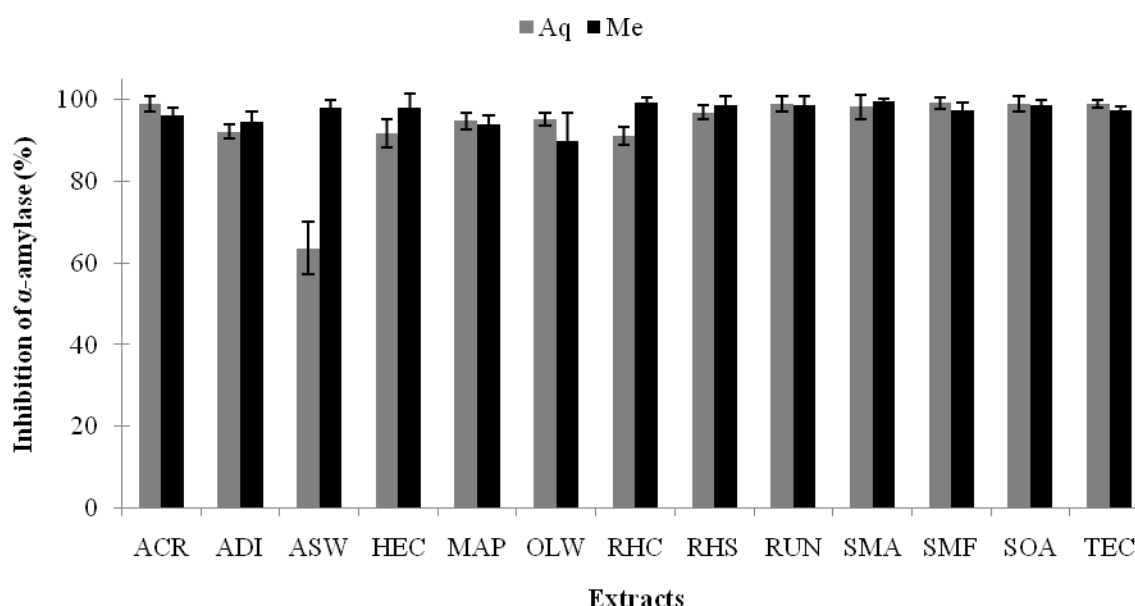


Figure 2. Percentage inhibition (mean \pm SD) of α -amylase activity by extracts of selected antidiabetic plants and acarbose (10 μg each). Note: Aq- aqueous, Me-Methanolic; ACR- Acarbose, ADI- *Adiantum incisum*, ASW- *Asterella wallichiana*, HEC- *Hedychium coronarium*, MAP- *Marchantia polymorpha*, OLW- *Oleandra wallichii*, RHC- *Rhus chinensis*, RHS- *Rhus succedanea*, RUN- *Rubus niveus*, SMA- *Smilax aspera*, SMF- *Smilax ferox*, SOA- *Sonchus arvensis*, TEC- *Tectaria coadunata*

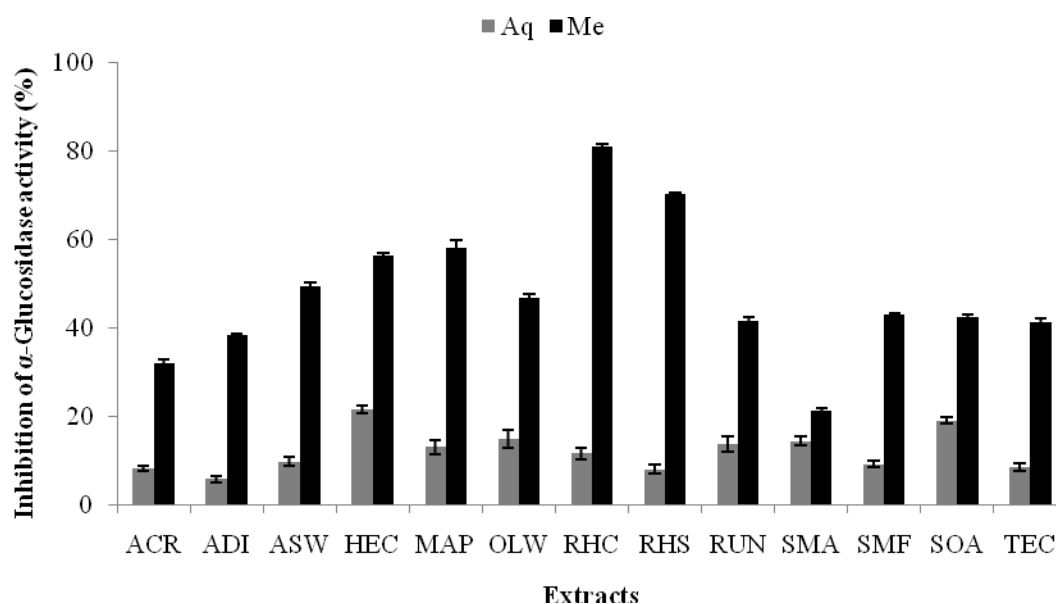


Figure 3. Inhibition of α -glucosidase activity by extracts (10 μ g) of selected Nepalese antidiabetic plants compared to that of Acarbose (50 μ g). Note: Aq- aqueous, Me- Methanolic; ACR- Acarbose, ADI- *Adiantum incisum*, ASW- *Asterella wallichiana*, HEC- *Hedychium coronarium*, MAP- *Marchantia polymorpha*, OLW- *Oleandra wallichii*, RHC- *Rhus chinensis*, RHS- *Rhus succedanea*, RUN- *Rubus niveus*, SMA- *Smilax aspera*, SMF- *Smilax ferox*, SOA- *Sonchus arvensis*, TEC- *Tectaria coadunata*

Discussion

α -Amylases are the enzymes of carbohydrate metabolism that convert complex carbohydrates like starch into simple oligosaccharides like maltose. Inhibition of α -amylases slows down the downstream processes that lead to the production and absorption of glucose in the intestine. Therefore, these are used as prime targets for lowering post-prandial glucose levels in diabetic patients. Bhandari et al. (2008) studied the antidiabetic potential of 50% methanolic extracts of 39 Nepalese medicinal plants in terms of inhibition of sucrase, rat intestinal maltase, and porcine pancreatic amylase. They selected 22 species with high inhibitory ability for these enzymes. They also reported least amylase inhibition activity (3.6%) in *Apium graveolens* and highest inhibition (93.5%) in *Bergenia ciliata*. The inhibition of porcine pancreatic amylase by extracts of species selected in present investigation is higher than that reported by Bhandari et al. (2008).

The high inhibition of porcine pancreatic amylase by the plant extracts of species in present investigation gives an indication that the antidiabetic/antihyperglycemic activities reported for extracts of species of genera like *Adiantum* (Saha et al. 2016; Paul et al. 2012), *Hedychium* (Kaur and Richa 2017; Pranitha et al. 2014), *Rubus* (Jouad et al. 2002; Alonso et al. 1980), *Smilax* (Rajesh and Perumal 2013), *Sonchus* (Sah et al. 2015) and other species used in present investigation is probably achieved through inhibition of α -amylase.

α -Glucosidase, being one of the key enzymes responsible for glucose production in the digestive system, is considered one of the potential targets in controlling post-prandial glucose levels in type 2 diabetic patients.

Therefore, glucosidase inhibitors are considered as a new hope in the struggle against diabetes. Well over 400 different compounds (61 terpenes, 37 alkaloids, 49 quinines, 103 flavonoids, 37 phenols, 73 phenylpropanoids, 8 sterides, and 43 different other compounds) with glucosidase inhibitory activity have been isolated from different groups of plants (Yin et al. 2014).

Plant extracts along with their active phytoconstituents from different species have been studied for their ability to inhibit α -glucosidase in vitro. Abd El-Wahab et al. (2013) reported *Berberis vulgaris* as one of the most promising plants with high inhibitory activities against α -glucosidase. Shim et al. (2003) also reported inhibition of α -glucosidase activity in aqueous extracts of *Rhus chinensis*. The inhibition of α -glucosidase activity in present study is comparable to that of Abd El-Wahab et al. (2013) only in the case of methanolic extracts of *Rhus chinensis* and *R. succedanea*. Since methanolic extract from bark of *Rhus javanica* (syn. *R. chinensis*) was reported to contain α -glucosidase inhibitors like Scopoletin and Phlorizin (Cho et al. 2013), high inhibition of α -glucosidase activity by methanolic extracts even in *R. succedanea* may possibly due to the presence of similar compounds. Moderate α -glucosidase inhibition activity by plant extracts of selected antidiabetic/antihyperglycemic plants used in present study indicates that the antidiabetic activities in these plants may involve other mechanisms in addition to α -glucosidase inhibition as their possible mode of action. Similarly, high degree of α -amylase inhibition in extracts of all the tested species suggests that the same mechanism possibly brings about the antidiabetic activities of these plants. Higher inhibition of the activities of enzymes of carbohydrate

metabolism, especially α -glucosidase by methanolic extracts may possibly be due to higher solubility of bioactive compounds in methanol.

Among the extracts of antidiabetic plants tested for their role in inhibiting α -amylase activity, all the extracts except the aqueous extract of *Asterella wallichiana* showed high inhibition of α -amylase comparable to that of standard acarbose irrespective of extraction medium (water or methanol). In the case of α -glucosidase, however, inhibition was very little in aqueous extracts and moderately high in methanolic extracts. All the species tested showed antidiabetic activity in vitro. These plants should be studied further for their role as antidiabetic/ antihyperglycemic substances in vivo and for search of novel inhibitors of α -amylase and α -glucosidase.

ACKNOWLEDGEMENTS

The authors are grateful to the Ministry of Education, Science, and Technology, Government of Nepal for financial support to carry out this research work. The authors would also like to thank Department of Plant Resources, Thapathali, Kathmandu for granting permission to collect plant samples from wild. The support provided by the Central Department of Botany and Central Department of Biotechnology, Tribhuvan University during this research is highly acknowledged.

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