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*Research*

# Anti-diabetic potential of crude extracts of medicinal plants used as substitutes for *Swertia chirayita* using *in vitro* assays

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## Abstract

*Swertia chirayita* is a highly traded medicinal plant of Nepal widely used for its anti-diabetic potential. In this study, two herbs (*Swertia nervosa* and *Andrographis paniculata*) often used as adulterants and substitutes of *Swertia chirayita* were analyzed for their antioxidant activity, *á*-glucosidase inhibitory potential and total phenolic content and compared with that of *Swertia chirayita.* Aqueous and 12% ethanolic extracts of the three herbs showed moderate to high antioxidant activity and moderate *á*-glucosidase inhibitory potential. HPLC/DAD revealed the presence of swertiamarin and mangiferin in all the *Swertia* species, while *Andrographis paniculata* contained cinnamates such as cinnamic acid, *p*-coumaric acid and chlorogenic acid. The antioxidant activity and *á*-glucosidase inhibitory potential was evident in the two herbs indicating their relevance as substitutes for *Swertia chirayita* for potential early stage management of type-2 diabetes and related complications.

**Key-words**: *Andrographis paniculata*, antioxidant activity, cinnamates, mangiferin, *Swertia nervosa*, swertiamarin, *á*-glucosidase, type-2 diabetes.

## Introduction

Over the past decade, herbal medicine has had more relevance in health care with repercussions for both global health and trade (Mahady 2001). About 25% of the drugs prescribed worldwide comes from plants, 121 such compounds are in current use (Rates 2001). A large proportion of the world's population (around 80%) use traditional medicine for their health care needs (Zirihi *et al.* 2005).

*Swertia chirayita* is a prized herb in countries like Nepal, India and China as a tonic (Bhargava *et al.* 2009). This plant is reported to possess hypoglycemic (Sellamuthu *et al.* 2009; Saxena *et al.* 1993), anti-carcinogenic (Saha *et al.* 2004), anti-hepatoxic (Karan *et al.* 1999), anthelmintic (Iqbal *et al.* 2006), anti-inflammatory (Banerjee *et al.* 2000), antipyretic (Bhargava *et al.* 2009) and antiviral (Verma *et al.* 2008) properties. Due to its medicinal importance and trade value *Swertia chirayita*, already critically endangered in India (CITES) and vulnerable in Nepal (IUCN), is being harvested indiscriminately from its wild habitat. The present situation calls for immediate efforts for the conservation of *Swertia chirayita*. Cultivation and sustainable harvesting are suitable options. One alternate way to decrease the harvesting of this species is using its substitutes. Many herbaceous species are traded as adulterants of *Swertia chirayita*, such as *Swertia ciliata, S. bimaculata, S. minor, S. nervosa, S. elegans, S. multiflora, S. lawii, S. densiflora* and *Andrographis paniculata* (Joshi and Dhawan 2005). Some of these species are also locally used as substitutes in the absence of *Swertia chirayita.* While plants of the genus *Swertia* (Family: Gentianaceae) are rich sources of biologically active phytochemicals, like xanthones, flavanoids, iridoid, secoiridoid glycosides and terpenoids (Pant *et al.* 2000), its substitute *Andrographis paniculata* (Family: Acanthaceae) contains flavonoids and

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diterpenoids (Li *et al.* 2007). This study aims to analyze and compare the phenolic-linked antioxidant and *á*-glucosidase inhibitory potential of *Swertia chirayita* and its substitutes *S. nervosa* and *Andrographis paniculata* in order to understand their relevance to early stage management of type-2 diabetes and its oxidative and hypertension-linked complications.

Diabetes mellitus long considered a disease of minor significance to world health is now taking its place as one of the main threats of human health in the 21st century (Zimmet *et al.* 2001). Since type-2 diabetes accounts for e"90% of all cases of diabetes worldwide, the current diabetes epidemic is attributed predominantly to rising cases of type-2 diabetes (Dagogo-Jack 2006). Type-2 diabetes is expected to increase 170% in developing countries over the period of 1995-2025 and this phenomenal increase has been related to the effect of westernization superimposed on latent genetic predisposition to diabetes (Dagogo-Jack 2006). Type-2 diabetes is connected to oxidative stress due to hyperglycemia and hyperlipidemia which induce inflammatory-immune responses and oxidative stress reactions, therefore the generation of free radicals accounts for aggravation of type-2 diabetes and cardiovascular complications (Baynes and Thorpe 1999; Pickup 2004). Postprandial hyperglycemia is the first metabolic abnormality occurring in type-2 diabetes (Lebovitz 1998) and plays an important role in the development of associated complications such as micro- and macro-vascular diseases (Baron 1998). *á*-Glucosidase catalyzes the final step of carbohydrate metabolism in biological systems (Liu *et al*. 2006). Inhibition of this enzyme is one of the ways to decrease postprandial blood sugar increase and manage type-2 diabetes. Plant phenolics are known for their *á*-glucosidase inhibitory potential and when combined with their antioxidant activity could be relevant in early stage management of type-2 diabetes. Dietary patterns characterized by higher intake of fruits, vegetables and whole grains are associated with reduced type-2 diabetes (Dembinska-Kiec *et al*. 2008). This is attributed to the presence of non-nutrient secondary metabolites especially phytochemicals such as phenolics and carotenoids which are known to possess antioxidant effects. Many medicinal plants including *Swertia chirayita* are known to have high concentration of phenolic compounds such as flavonoids, xanthones and iridoids which are potent antioxidants. The effect of antioxidants and its relation to type-2 diabetes is well documented (Dembinska-Kiec *et al*. 2008; Sabu and Kuttan 2002).

## Materials and Method

### REAGENTS

Rat intestinal *á*-glucosidase (EC3.2.1.20), 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, chlorogenic acid, *p*-coumaric acid and cinnamic acid were purchased from Sigma Chemical Co (ST. Louis, MO) and the standards mangiferin, swertisin, swertiamarin and amarogentin were purchased from Chromadex ™(Irvine, CA).

### PLANT SAMPLES

*Swertia chirayita* and *Swertia nervosa* were collected from Sindhupalchok, Marming at the end of the flowering season in late August to October 2007 when the plants were in the seed dispersal phase. *Andrographis paniculata* was collected from a local trader, Ason, Kathmandu, Nepal.

### SAMPLE EXTRACTION

Individual plant samples were divided into root, shoot and mixture of inflorescence with leaf (IL) and shade dried at room temperature of 20-25ºC. These different plant parts were then ground using coffee grinder (Mr. Coffee, Sunbeam Products Inc., Boca Raton, FL). Two g of the dried plant sample was mixed with 100 mL of either water or 12% ethanol (to make 2%, w/v extract). This mixture was left at room temperature (25ºC) for 12 h (overnight). The sample was then filtered the next day using Whatman No. 1 filter paper. Locally, *S. chirayita* is taken as an infusion by steeping the powdered whole plant over night. The extraction process for this study simulates this commonly used method in therapy.

### TOTAL PHENOLICS ASSAY

The total phenolics in all samples were determined by using a method modified by Shetty *et al.* (1995). Sample extract (0.5 mL) was added to a test tube and mixed with 0.5 mL of 95% ethanol and 5 mL of distilled water. To each sample, 0.5 mL of 50% (v/v) Folin-Ciocalteu reagent was added and mixed with 1 mL of sodium carbonate (5%w/v) and left in the dark for 1 h. The absorbance was read at 725 nm using a spectrophotometer (Genesys UV/Visible, Milton Roy, Inc., Rochester, NY). Different concentrations of gallic acid were used to develop a standard curve. Results were expressed as mg of gallic acid equivalent (GAE)/g sample dried weight (DW).

### ANTIOXIDANT ACTIVITY BY THE DPPH RADICAL INHIBITION ASSAY

The antioxidant activity was determined by the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical-scavenging method modified from Kwon *et al.* (2006). A 250 ìL aliquot of the sample extract was mixed with 1250 µL of DPPH (60 ì M in ethanol). The absorbance was measured after 5 min at 517 nm using a spectrophotometer (Genesys UV/Visible, Milton Roy, Inc., Rochester, NY). The readings were compared with the controls, containing 95% ethanol instead of sample extract. The percentage inhibition (%) was calculated by:

$$\% \text{ inhibition} = \frac{\text{Abs (absolute) control} - \text{Abs extract}}{\text{Abs control}} \times 100$$

### $\alpha$-GLUCOSIDASE INHIBITION ASSAY

The assay was performed according to Worthington Enzyme Manual (Worthington 1993), with some modifications (McCue *et al.* 2005). Alpha-glucosidase was assayed by using 50 ì L of sample extract and 100 ìL of 0.1 M phosphate buffer (pH 6.9) containing $\alpha$-glucosidase solution (1 U/mL) and was incubated in 96 well plates at 25°C for 10 min. After pre-incubation, 50 ìL of 5 mM *p*-nitrophenyl-$\alpha$-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25°C for 5 min. Before and after incubation, absorbance readings were recorded at 405 nm by microplate reader (Thermomax, Molecular Device Co., Sunnyvale, CA) and compared to a control which had 50 ìL of buffer solution in place of the extract. The $\alpha$-glucosidase inhibitory activity was expressed as % inhibition and was calculated as above.

### HPLC ANALYSIS OF PHENOLIC PROFILES

Two mL of the extracts were filtered through a 0.2 ìm filter and 5 ìL were injected in a HPLC agilent 1100 series equipped with auto-sampler and DAD 1100 diode array detector (Agilent Technologies, Palo Alto, CA). The solvents used for gradient elution were (A) 10 mM phosphoric acid (pH 2.5) and (B) 100% methanol. The methanol concentration was increased to 60% for the first 8 min and to 100% over the next 7 min, then decreased to 0% for the next 3 min and was maintained for the next 7 min (total run time, 25 min). The analytical column used was Agilent Zorbax SB-C18, 250 mm x 4.6 mm i.d., with packing material of 5 ìm particle size at a flow rate of 1 mL/min at ambient temperature. During each run the absorbance was recorded at 225 nm and 306 nm and the chromatogram integrated using Agilent Chemstation enhanced integrator. Calibration was performed by injecting the standards of amarogentin, swertisin, chlorogenic acid, *p*-coumaric acid, cinnamic acid, mangiferin and swertiamarin at different concentrations. Peak identification was performed by comparison of retention times and diode array spectral characteristics with these standards and other standards already present in the library.

### STATISTICAL ANALYSIS

Data were analyzed using one-way analysis of the variance (ANOVA). Post-hoc comparisons were carried out using LSD test or planned comparison done in Statistic Software Package, version 5.0 (StatSoft, Inc., Tulsa, OK, USA).

## Results

### TOTAL PHENOLICS AND ANTIOXIDANT ACTIVITY

Highest total phenolic content was found in aqueous and ethanolic extract of *S. chirayita* (both 5.6 mg/g GAE -Gallic Acid Equivalent, respectively), followed by *S. nervosa* (both 4.7 mg/g GAE, respectively) and *Andrographis paniculata* (4.5 mg/g GAE, respectively) (Fig. 1). Aqueous and ethanolic extracts of *Swertia chirayita*, *S. nervosa* and *Andrographis paniculata* did not show significant differences ($p > 0.05$) in total phenolic content. Although in general, ethanolic extracts had slightly higher total phenolic content than aqueous extracts, there was no significant difference between total phenolic content present in aqueous and ethanolic extracts of all the species ($p > 0.05$).

In this study, highest DPPH radical scavenging activity was present in aqueous and ethanolic extracts of *S. chirayita* followed by extracts of *S. nervosa* and *Andrographis paniculata* (Fig. 2). There were significant differences in DPPH radical scavenging activity of both aqueous and ethanolic extracts among all the three species ($p < 0.05$).

### $\alpha$-GLUCOSIDASE INHIBITORY POTENTIAL

Ethanolic extracts of *S. nervosa* had the highest $\alpha$-glucosidase inhibitory potential (22.6 % inhibition) followed by ethanolic extract of *Andrographis paniculata* (20.6 % inhibition) and *S.*

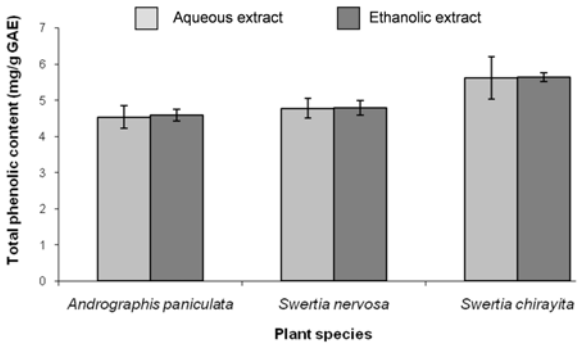


**Figure 1**. Total phenolic content (mg/g GAE) of aqueous and ethanolic extracts of *Andrographis paniculata*, *Swertia nervosa* and *S. chirayita*.

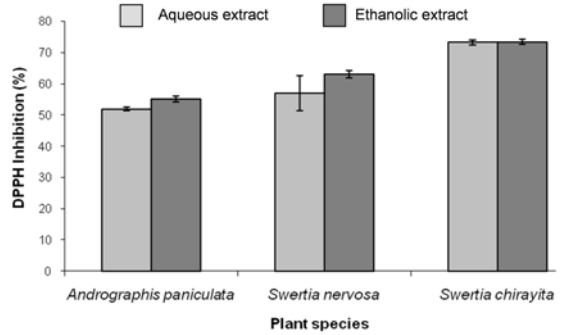


**Figure 2**. DPPH radical scavenging activity (% inhibition) of aqueous and ethanolic extracts of *Andrographis paniculata*, *Swertia nervosa* and *S. chirayita*.

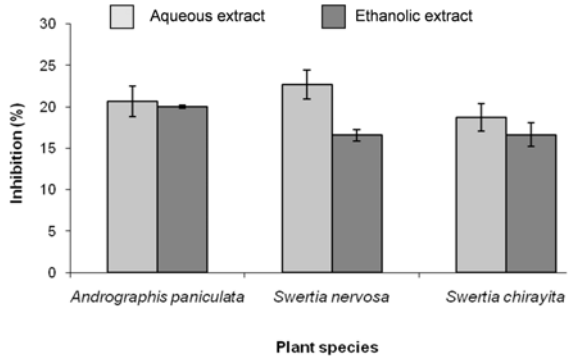


**Figure 3**. Inhibition of α-glucosidase (%) by aqueous and ethanolic extracts of *Andrographis paniculata*, *Swertia nervosa* and *S. chirayita*.

*chirayita* (18.7 % inhibition) (Fig. 3). All the extracts showed dose dependent α-glucosidase inhibitory potential (Fig. 4). There was no significant difference in α-glucosidase inhibitory potential between aqueous and ethanolic extracts of *S. nervosa*, *Andrographis paniculata* and *S. chirayita* ($p>0.05$). There was negative to high correlation between α-glucosidase inhibitory potential and total phenolic content. In general, there was low correlation between antioxidant activity and α-glucosidase inhibitory potential in all extracts.

### HPLC ANALYSIS

The phytochemicals present in the aqueous and ethanolic extracts of the *Andrographis paniculata, S. nervosa* and *S. chirayita* were analyzed using HPLC/DAD. The two species of *Swertia* contained swertiamarin and mangiferin. Amarogentin was detected in *S. chirayita* (Figures 5a), but not in *S. nervosa* (Fig. 5b). Swertisin was detected in *S. nervosa* which was absent in *S. chirayita*. The highest quantity of swertiamarin was present in ethanolic extracts of *S. chirayita* and *S. nervosa* (both 0.18 mg/g DW, respectively). Similarly, highest quantity of mangiferin and amarogentin was found in the ethanolic extracts of these two species. The extracts of *Andrographis paniculata* on the other hand showed the presence of cinnamates such as chlorogenic acid, *p*-coumaric acid and cinnamic acid and their derivatives (Fig. 5c). In general, all the ethanolic extracts had higher amount of phytochemicals than the aqueous extracts. Chlorogenic acid and their derivatives were highest in aqueous and ethanolic extracts of *Andrographis paniculata* (both 0.04 mg/g DW respectively).

## Discussion

Formation of reactive oxygen species is a natural metabolic occurrence but if left unchecked these radicals can attack the structural and genetic apparatus of the cell (Harris 1992) causing oxidative stress. Evidences have indicated that oxidative stress may facilitate the progressive impairment of â-cell function in the pathogenesis of type-2 diabetes (Ceriello and Motz 2004). It has been suggested that diabetic patients with defective cellular antioxidant against the oxidative stress generated by hyperglycemia can predispose the patient to organ failure and therefore antioxidant therapy has been suggested to have benefit for these patients (Ceriello 2003). In the present study, aqueous and ethanolic extracts of *S. chirayita* showed highest antioxidant activity DPPH radical scavenging activity and high total phenolic content followed by *S. nervosa* and *Andrographis paniculata*. There was also moderate to high correlation between DPPH radical scavenging activity and total phenolic content of all extracts. Phenolics are secondary metabolites of plants and are the

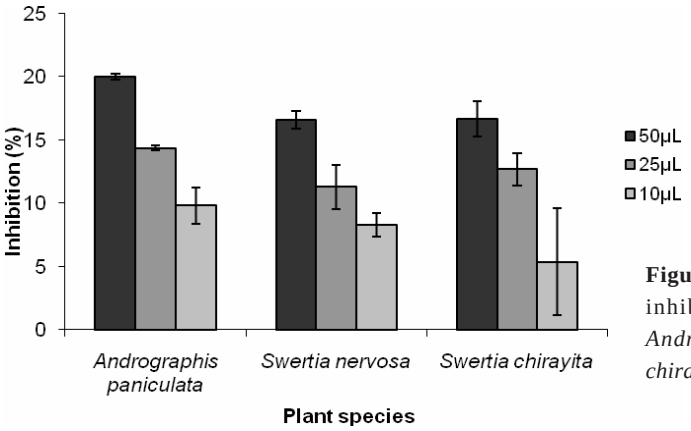


**Figure 4**. Dose-dependent changes in $\alpha$-glucosidase inhibitory potential (%) by aqueous extracts of *Andrographis paniculata*, *Swertia nervosa* and *S. chirayita*.

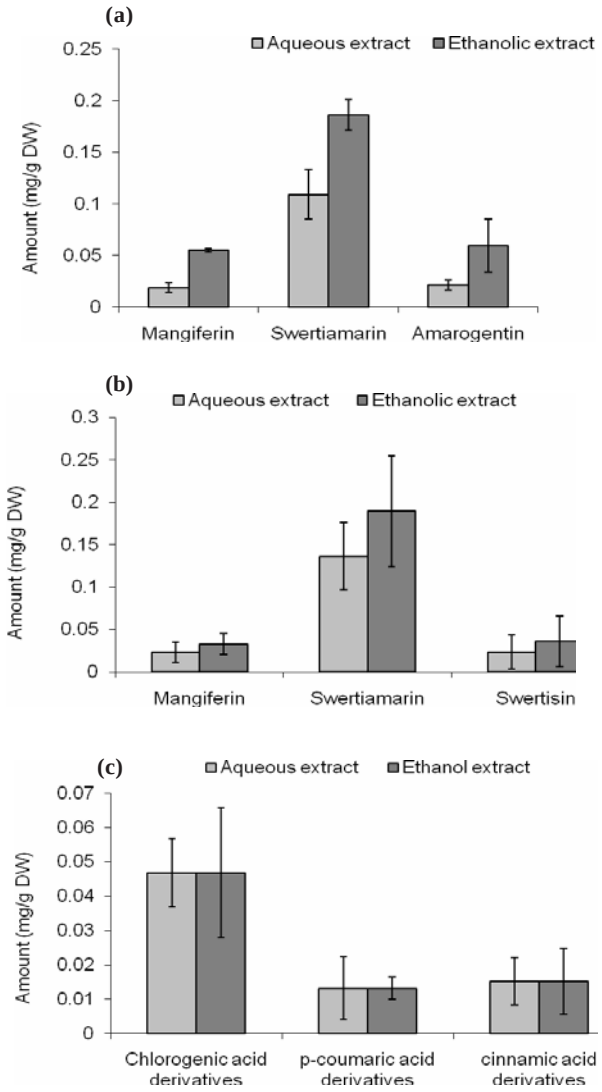


**Figure 5**. Phytochemicals (mg/g DW) present in aqueous and ethanolic extracts of (a) *Swertia chirayita*, (b) *Swertia nervosa*, and (c) *Andrographis paniculata*.

most abundant antioxidants present in our food and many medicinal plants (Parr and Bolwell 2000). These compounds exhibit many significant biological functions, including protection against oxidative stress and degenerative diseases such as type-2 diabetes (Han *et al.* 2007).

Regulation of postprandial glucose by regulating starch digestion and reducing active transport of glucose across intestinal brush border membrane is one of the mechanisms of reducing the risk of type-2 diabetes. Prevention of post-prandial hyperglycemia is important because it is the earliest abnormality of glucose homeostasis associated with type-2 diabetes. It is also associated with elevated glycosylated haemoglobin and contributes to associated complications such as retinopathy, and micro- and macro- vascular complications (Baron 1998; Stratton *et al.* 2000). Alpha-glucosidase inhibitors have wide applications as clinical treatment of carbo-hydrate mediated diseases, such as type-2 diabetes and obesity (Liu *et al.* 2006). The anti-diabetic potential of *S. chirayita* is reportedly due to the phytochemical swerchirin (1,8-dihyroxy-3,5-dimethoxyxanthone) which induced the release of insulin from â-cells of islets of langer-haans of the pancreas (Sekar *et al.* 1987, Saxena *et al.* 1993). However, locally *S. chirayita* is taken as an infusion and although there may be other mechanisms responsible for its antidiabetic potential which need further *in vitro* and *in vivo* studies. The results of this study suggest that the anti-diabetic potential of crude extracts of *S. chirayita* and its substitutes used in traditional healing in Nepal could be linked to their $\alpha$-glucosidase inhibitory potential and phenolic-linked antioxidant activity.

In the present study, mangiferin and swertiamarin was found in both species of *Swertia*. While amarogentin was present in *S. chirayita*, swertisin was found in *S. nervosa*.

Mangiferin, a C-glucosylxanthone, is reported to possess considerable potential for hypoglycemic (Muruganandan *et al.* 2005), antioxidant (Sanchez *et al.* 2000), antiviral (Zheng and Lu 1990), antiatherogenic (Muruganandan *et al.* 2005), immunodialtory (Guha *et al*. 1996), anti-proliferative, immunodilatory, cardiotonic and diuretic properties (Andreu *et al.* 2005). Swertiamarin, a secoiridoid glycoside, has been reported to possess hepatoprotective and antiedematogenic/ anti-inflammatory, free radical scavenging, cardio-protective, anti-atherosclerotic (Vaidya *et al*. 2009), anti-bacterial (Kumarasay *et al.* 2003), anticholinergic (Suparna *et al.* 1998), antinociceptive (Jaishree *et al.* 2009) and antispastic potential properties (Vaijanathappa and Badami 2009). Swertisin, a *c*-glycosidic flavonoid, has been reported to have significant activity against sugar induced cataract *in vitro* due to its aldose reductase inhibitory potential (Patel and Mishra 2009). Amarogentin, a secoiridoid, is a known topoismerase inhibitor (Ray *et al*. 1996), chemopreventive with potential anti-leishmanial (Medda *et al*. 1999) and gastroprotective properties (Niiho *et al.* 2006). The combined properties (such as antihypoglycemic, antilipidemic, antiatherogenic, cardioprotective, immunodilatory, cardiotonic, anti-inflammatory, analgesic, antioxidant, gastroprotective, hepatoprotective, anticholerogenic) of the three phytochemicals (mangiferin, amarogentin and swertiamaria) present in *S. chirayita* may be responsible for its therapeutic action against type-2 diabetes and its complications.

In India, *Andrographis paniculata* is widely used for various ailments and is also known by the same trade name 'chiretta' or 'chiraita' as *S. chirayita*. This has been a source of confusion for many medicinal plant traders and practitioners. Due to similarities in their therapeutic actions, *Andrographis paniculata* is suggested as a substitute for *S. chirayita* (Girach *et al.* 1994). While their therapeutic actions may be similar, the main phytochemicals reported in *Andrographis paniculata* are kalmeghin; diterpes: andrographolide, andrographiside, neoandrographolide as well as panicolide, caffeic acid, chlorogenic acid and other polyphenolics (Li *et al.* 2007; Hossain *et al.* 2007). In our study, cinnamic acid along with chlorogenic acid and *p*-coumaric acid and their derivatives were present in both aqueous and ethanolic extracts of *Andrographis paniculata*. Cinnamates such as chlorogenic acid, *p*-coumaric acid and cinnamic acid are potent antioxidants (Miller and Rice-Evans 1997). Cinnamic acid and chlorogenic acid is also reported to have anti-diabetic potential (Lakshmi *et al.* 2009; McCarty 200). *Andrographis paniculata* has been reported to show antioxidant, antioedema, analgesic (Lin *et al.* 2009), anti-cancer (Matsuda *et al.* 1994), and anti-inflammatory activities. It is also used to relive gripping, irregular stools, loss of appetite, and as a febrifuge, tonic alternative and anthelmintic medicine (Sheeja *et al.* 2006). *Andrographis paniculata* is also reported to show potential antidiabetic activity (Hossain *et al.* 2007).

The results of this study indicate that both aqueous and ethanolic extracts of *S. chirayita* and its adulterants: *S. nervosa* and *Andrographis paniculata* have medium to high antioxidant activity and α-glucosidase inhibitory potential. Combined with other therapeutic properties that have been reported, these medicinal herbs may help in counteracting the related complications of type-2 diabetes. Based on these *in vitro* assays, *S. nervosa* and *Andrographis paniculata* could potentially be used as the substitutes of *S. chirayita* for early stage management of type-2 diabetes and its complications. Further *in vivo* and clinical studies are required to understand its significance and wider applications in therapy

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