

Antioxidant, Antiglycation Potentials and Total Phenolic Content of *Scoparia dulcis* Decoction

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Abstract

Long term diabetes mellitus increases the risk of many micro and macro vascular diabetic complications and oxidative stress, due to formation of excessive amounts of free radicals. Non enzymatic protein glycation is the key molecular basis of the above complications observed in diabetic individuals. The current tendency to use herbal treatments in diabetes mellitus is mainly due to the therapeutic efficacy, safety, low cost and minimal adverse effects. Among the many herbs used, the decoction prepared using the whole plant of *Scoparia dulcis* is a frequently used traditional medicine in the treatment of diabetes mellitus.

Since no data are available on the antiglycation potential of the decoction of *S. dulcis* plant this study focused on determining the antiglycation potential, DPPH and ABTS antioxidant potentials and total phenolic content of the *S. dulcis* decoction.

The commercially available dried *S. dulcis* and three other fresh samples collected from three different areas were used for the study. The DPPH and ABTS antioxidant potentials of the samples were in the range of 450-540 $\mu\text{g mL}^{-1}$ and 549-615 TEAC mmol g^{-1} respectively. Antiglycation potentials of the four samples were in the range of 131-230 $\mu\text{g mL}^{-1}$ and total phenolic contents were 160-186 mg/GAE/g. The results of the study prove the effectiveness of the use of *S. dulcis* plant in traditional medicine for diabetes.

Keywords: S. dulcis, decoction, antiglycation, antioxidant, phenolic contents, traditional medicine

1. Introduction

Decoction prepared with *Scoparia dulcis*, a perennial herb (sweet broom weed) is documented as a traditional treatment in managing diabetes mellitus. Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism characterised by increased fasting and post prandial blood sugar levels. According to International Diabetes Federation (4th edition, 2009), 285 million among the seven billion of total world population is suffering from diabetes mellitus. Though pathophysiology of diabetes remains to be fully understood, experimental evidences suggest the involvement of free radicals in the pathogenesis of diabetes (Matteucci et al., 2000) and more importantly in the development of diabetic complications (Lipinski et al., 2001). Free radicals generated during various metabolic process in the body leads to oxidative stress and play a pivotal role in the development of both micro-and macro vascular diabetes complications. The metabolic abnormalities of diabetes cause mitochondrial superoxide overproduction in endothelial cells of both large and small vessels, as well as in the myocardium. Free radicals are capable of damaging cellular molecules, DNA, proteins and lipids leading

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to altered cellular functions. During diabetes, lipoproteins are oxidised by free radicals. There are also multiple abnormalities of metabolism in very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL) in diabetes. Lipid peroxidation is enhanced due to increased oxidative stress in diabetic condition. Many recent studies reveal that antioxidants capable of neutralizing free radicals are effective in preventing experimentally induced diabetes in animal models (Naziroglu et al., 2001) as well as reducing the severity of diabetic complications. Apart from this, advanced glycation end products (AGEs) are formed by nonenzymatic glycosylation of proteins in diabetes mellitus. AGEs tend to accumulate on long-lived molecules in tissues and generate abnormalities in cell and tissue functions (Elgawish et al., 2012). In addition, AGEs also contribute to increased vascular permeability in both micro- and macro vascular structures by binding to specific macrophage receptors. This results in formation of free radicals and endothelial dysfunction. AGEs are also formed on nucleic acids and histones and may cause mutations and altered gene expression. Several studies on the antidiabetic activity of aqueous fraction of *S. dulcis* showed significantly improved specific insulin binding in streptozotocin induced male Wistar rats (Pari et al., 2004). An aqueous extract of *S. dulcis* showed marked *in vitro* antioxidant activity, which supports the therapeutic effects claimed by traditional practitioners (Rathnasooriya et al., 2005). The present study aimed to determine the antioxidant and antiglycation activities of the decoctions prepared according to Ayurveda, using a commercially available dried sample of *S. dulcis* herb available in the traditional herbal market and samples collected as fresh, from three different areas in the country where they grow profusely and dried under laboratory conditions. As plant derived phenolic compounds are highly accountable for antidiabetic activities, the total phenolic content of the decoctions were also determined.

2. Materials and Methods

2.1 Collection of the plant materials

The commercially available dried samples of the plant material was purchased from a reputed vender of herbal materials from the traditional herbal market in Colombo, Sri Lanka and fresh samples were collected from Bandarawela, Wijerama and Rukmale areas in Sri Lanka.

2.2 Identification of plant materials

Identities of the specimens of plant materials collected were authenticated by the Botanist at Bandaranaike Memorial Ayurveda Research Institute, Nawinna, Maharagama, Sri Lanka.

2.3 Preparation of plant materials

Fresh and commercially available dried samples were air dried for 24 hours at room temperature. Fresh samples were further dried in a dehydrator (Leader, India) at 55° C for 24 hours and powdered using a domestic grinder (National, Japan[®]) to obtain powdered samples (2,000 µm) and were packed in polyethylene bags and stored in air tight containers at -4° C in a cold room until used for analyses.

2.4 Preparation of decoctions

Water extracts of dried and milled powdered samples were prepared according to the traditional method practiced in Ayurveda medicine to prepare ‘Kasaya’. Each powdered sample (60 g; similar weight of 12 ‘kalan’) was boiled with 960 mL of water (4 ‘patha’) over a low flame until concentrated to 240 mL (1‘patha’) and filtered through a silk cloth (500 µm). The filtrate was freeze dried (Feyela, FDU-1,200) and samples were stored at -4° C in a cold room in air tight containers.

2.5 Determination of antioxidant activity

a) ABTS assay

2,2’-Azinobis (3-ethyl benzothiazoline-6-sulphonic acid) diammonium salt (Sigma, USA[®]) radical cation decolorization assay was used to measure the antioxidant activity (Miller et al., 1996) with

slight modifications. The stock solutions of 7 mM ABTS and 2.4 mM potassium per sulphate were mixed in equal parts to prepare the working solution. ABTS⁺ solution was prepared by diluting 1.0 mL ABTS⁺ solution with 16.0 mL phosphate buffer (pH 7.2) to obtain an initial absorbance of 0.700±0.01. Freeze dried water extract of each sample was prepared in 3 concentrations by dissolving in phosphate buffer solution and the reaction mixture was prepared mixing 100 µL of plant extract with 3.0 mL ABTS⁺ solution. ABTS⁺ radical scavenging activity was determined after measuring the fall of absorbance exactly after 15 min. Trolox (6-Hydroxy 2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as the standard and the blank sample was prepared by adding 100 µL phosphate buffer to 3.0 mL ABTS⁺ solution. Data are reported as mean±SD of the three replicates as Trolox equivalents using the equation obtained from the calibration curve of Trolox.

b) DPPH assay

Assay was carried out with DPPH (2,2 diphenyl-2-picryl hydrazyl hydrate) (Sigma, USA[®]) using a spectrophotometric method (Brand Williams et al., 1995). Freshly prepared DPPH solution was used for each experiment. Reaction mixture was prepared using 2.5 mL of 6.5×10⁻⁵ M DPPH solution and 0.5 mL of sample dissolved in methanol and a control sample with 2.5 mL of 6.5×10⁻⁵ M DPPH solution and 0.5 mL of methanol (Sigma, USA[®]). Samples were tested in five concentrations and each sample was assayed in triplicate. All samples were incubated at room temperature for 30 minutes in dark and then absorbance was measured at 540 nm using UV-Vis spectrophotometer (SHIMADZU UV mini 1240[®]). The percentage of DPPH radical scavenging activity was determined in five concentrations using the equation 1. BHT (Butyl Hydroxy Toluene) was used as the reference standard.

$$\% \text{ scavenging activity} = A_0 - A_s / A_0 \times 100 \quad (1)$$

where: A_s-Absorbance of the DPPH solution of the control sample

A₀-Absorbance of the DPPH solution in the presence of plant extract

The sample concentration which gives 50% scavenging activity was estimated as IC₅₀ value from regression analysis using Minitab 14.

2.6. Determination of antiglycation activity

Antiglycation activity was determined using the Bovian Serum Albumin (BSA) assay (Matsuura et al., 2002) with slight modifications. In all experiments the final reaction volume was 1.0 mL and carried out in 1.5 mL Eppendorf tubes. Bovian serum albumin 500 µL (1 mg mL⁻¹ concentration) was incubated with glucose 400 µL (500 mM final concentration) and 100 µL sample, 100 µL phosphate buffer saline (PBS) instead of the sample, as the sample control and 100 µL Arbutin (Sigma-Aldrich, USA[®]) as the reference standard. Negative control was carried out at the same time with BSA 500 µL (1 mg mL⁻¹ concentration), 400 µL phosphate buffer saline (pH 7.4) and 100 µL sample incubated under same conditions. The reaction was allowed to proceed at 60° C for 24 hours, thereafter terminated the reaction by adding 10 µL of 100% (W/V) trichloroacetic acid (TCA). The TCA added mixture was kept at 4° C for 10 minutes and centrifuged 4 minutes at 13,000 rpm. The precipitate was dissolved with alkaline PBS (pH 10) and quantified for the relative amount of glycated BSA based on fluorescence intensity by Fluorescent Micro Plate Reader (Spectra Max Gemini EM[®]). The excitation and emission wavelengths used were at 370 nm and 440 nm respectively. Each sample was assayed in five concentrations and in triplicate. Percentage of inhibition was calculated using the equation 2 and the sample concentration required for the 50% of inhibition was calculated using Minitab 14.

$$\% \text{ of inhibition} = OD \text{ blank} - (OD \text{ sample} - OD \text{ sample negative}) / OD \text{ blank} \times 100 \quad (2)$$

where: OD-Optical density

2.7 Determination of total phenolic content

The total phenolic content of each extract was determined spectrophotometrically using the Folin-Ciocalteu reagent (Slinkard et al., 1977). This test is based on the oxidation of phenolic groups by phosphomolybdic and phosphotungstic acids (FC reagent) which yield a blue color with a broad maximum absorption at 765 nm. Freeze-dried sample (0.01 g) from each plant was extracted with 5.0 mL of hot 70% methanol and keeping in a water bath at 70° C for 10 minutes after mixing using a vortex mixer. Sample was cooled to room temperature (30° C) and centrifuged at 3,500 rpm (Thermo Scientific®). The supernatant was decanted. Extraction was repeated twice following the same procedure. All extractions were pooled and diluted up to the required volume using 70% methanol. The reaction mixture was prepared using 0.5 mL of extracted sample with 2.5 mL of Folin-Ciocalteu reagent (Sigma-Aldrich, USA®) which was diluted 10 times using 70% methanol. Sodium carbonate (Sigma-Aldrich, USA®) 7.5% (w/v) solution was added after three minutes to the above mixture and kept at 45° C for 10 minutes in an incubator (Microsil, India®). Absorbance of each plant extract and prepared blank by adding 0.5 mL of methanol instead of the plant extract to the above mixture was measured at 765 nm using the UV-Visible spectrophotometer (SHIMADZU UV mini 1240®).

Total phenolic content was expressed as mg Gallic acid equivalent/g using the standard curve equation $y=0.633x+0.0237$, $R^2=0.9979$, obtained from the calibration curve for Gallic acid. Data are expressed as mean±SD of three replicates.

2.8 Statistical analysis

All experiments were carried out in triplicate and presented as mean±standard error (SE) using Minitab 14. One way analysis of variance (ANOVA) and Tukey's multiple comparisons were carried out to test for any significant difference between the means. The level of statistical significance was set at $p<0.05$.

3. Results

3.1 DPPH antioxidant potentials of *S. dulcis* decoctions

The DPPH activities of the commercial sample (490±2.1) and laboratory dried samples from Bandarawela, and Rukmale (437±2, 450±1.8) were significantly different but were in the range of 450-490 µg/mL. Sample from Wijerama had low activity (Figure 1) Butylated hydroxy toluene (BHT) used as the reference expressed 18.5±1.2 µg/mL activity. DPPH antioxidant potential is inversely proportional to the sample concentration.

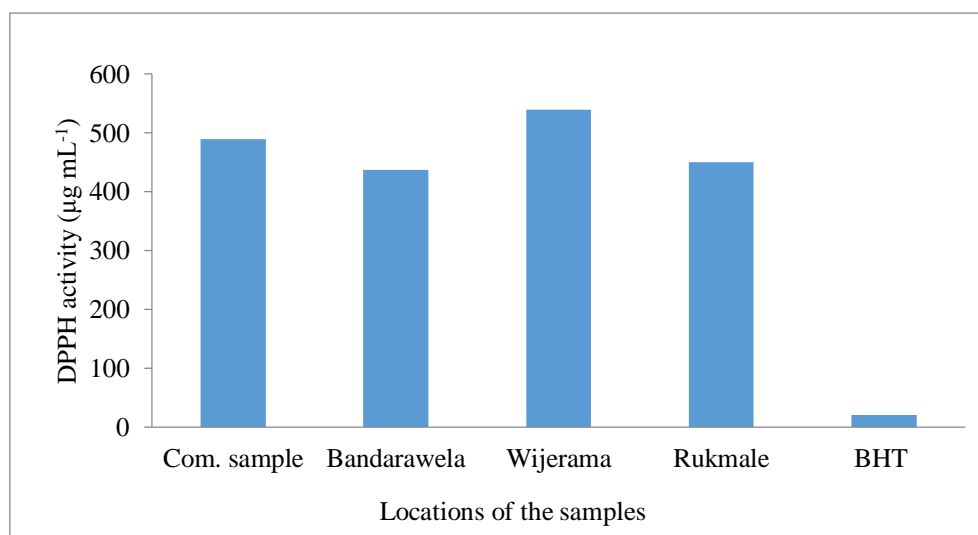


Figure 1. DPPH antioxidant potentials of *S. dulcis* decoction.

3.2 ABTS antioxidant potentials of *S. dulcis* decoctions

The highest ABTS activity of 615 TEAC mmol g⁻¹ was shown by the commercial sample (Figure 2) and was significantly different (p<0.05) from other three samples collected fresh and dried under laboratory conditions (549, 553, 550 TEAC mmol g⁻¹). The ABTS activities of those three samples were not significantly different.

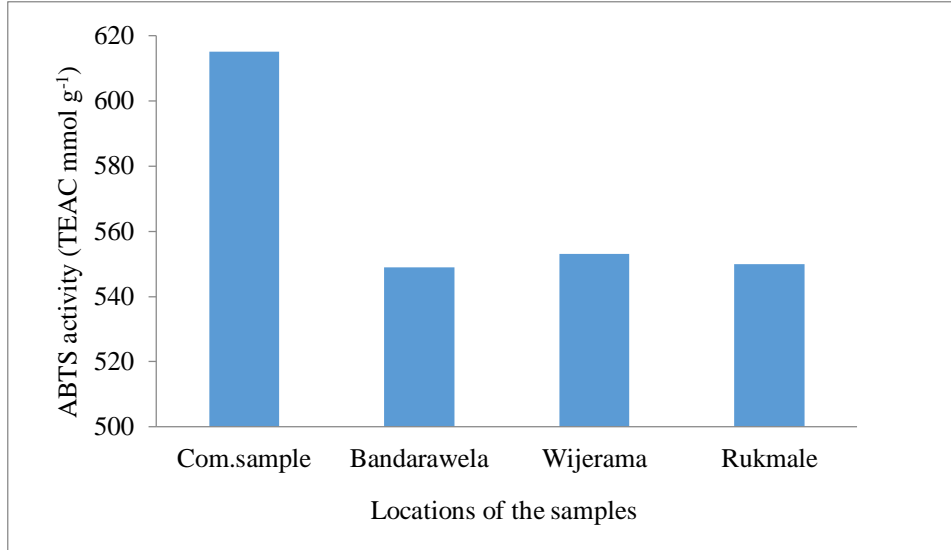


Figure 2. ABTS antioxidant potentials of *S. dulcis* decoction.

3.3. Antiglycation potentials of *S. dulcis* decoction

In humans, serum proteins with slow turnover rates that are exposed to high concentrations of glucose are particularly susceptible to non-enzymatic glycation. In the *in vitro* study Bovian Serum Albumin (BSA) was used as it is the most abundant protein in serum. The sample collected from Bandarawela area showed significantly high activity (131±1.3 µg mL⁻¹). The antiglycation activities of the commercial sample and samples from Rukmale and Wijerama (196 µg mL⁻¹, 213 µg mL⁻¹, and 230 µg mL⁻¹ respectively) were significantly different (p<0.05) (Figure 3). The reference, Arbutin showed an activity of 70.3 µg mL⁻¹. Antiglycation potential is inversely proportional to sample concentration.

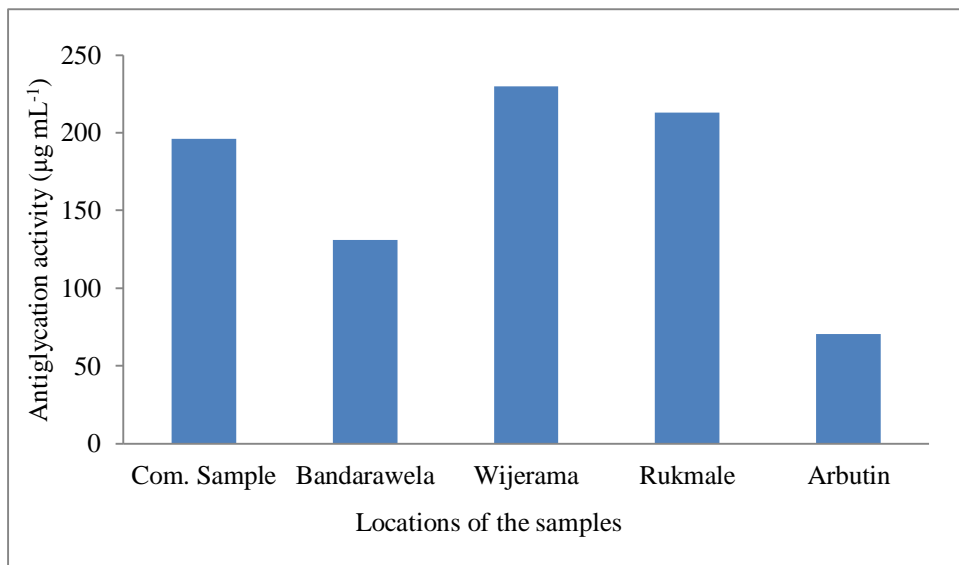


Figure 3. Antiglycation potential of *S. dulcis* decoction.

3.4 Total phenolic content of *S. dulcis* decoction

In this study the total phenolic content of the sample collected from Wijerama (Figure 4) contained the highest phenolic content (186 mg/GAE/g) while the commercial sample and samples collected from Bandarawela and Rukmale were not significantly different (160, 167, 170 mg/GAE/g) respectively.

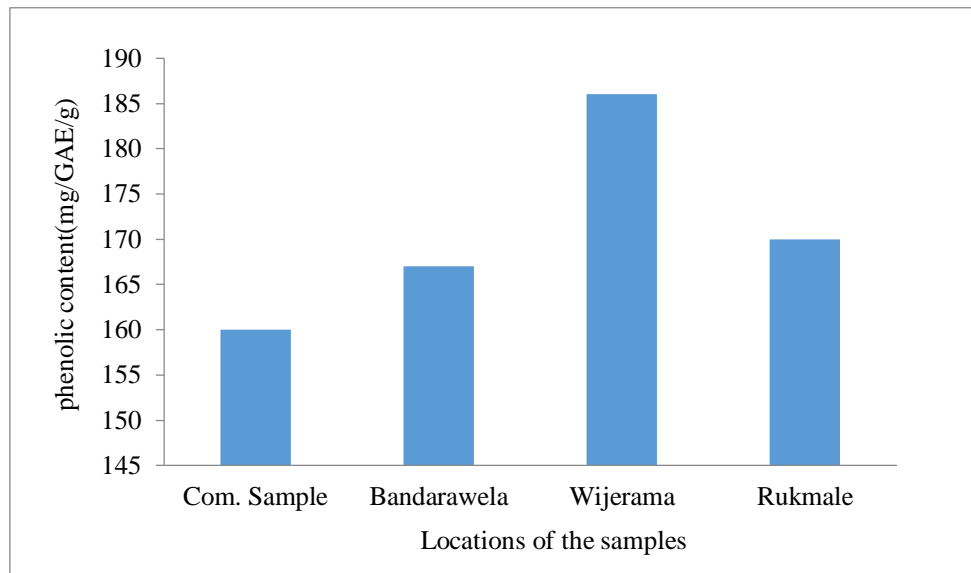


Figure 4. Total phenolic content of *S. dulcis* decoctions.

4. Discussion

Antioxidant activity of medicinal plants is mainly due to the presence of secondary metabolites (El-toumy et al., 2011). The scientific literature reveals numerous chemical studies on the herbs and isolated chemical constituents including coumarins, phenols, saponins, tannins, amino acids, flavonoids, terpenoids and catecholamines (Rathnasooriya et al., 2005). Phytochemical screening has shown that *S. dulcis* contains diterpenoids, flavonoids (Ramesh et al., 1979), tannins, alkaloids, triterpenes, hexacosanol, β -sitosterol, ketones, dulcitone and amellin an antidiabetic compound (Hayashi, 1998). Phenolic compounds possess ideal structure for free radical scavenging activities since they have phenolic hydroxyl groups that are prone to donate a hydrogen atom or an electron to a free radical and extend conjugated aromatic system to delocalize an unpaired electron. For phenolic acids and their esters, the reduction activity depends on the number of free hydroxyl groups in the molecule, which would be strengthened by steric hindrance (Dziedzic et al., 1983). In this study the decoctions of *S. dulcis* contained significant amount of total phenolic content.

According to the findings the plant *S. dulcis* contains high ABTS and DPPH potentials and high phenolic contents. A similar study was carried out by Rathnasooriya et al. (2005) and have reported that the aqueous extract of *S. dulcis* possesses antidiabetic and antioxidant properties. Give specific information in the study of the hypoglycemic activity of methanol extract of *S. dulcis* of Mishra et al. (2013) has reported very promising result on the antioxidant activity in DPPH assay and correlate the antioxidant potential with the antidiabetic activity. The decoction use for the study also expressed significant antioxidant activities in both assays. Free radical formation occurs in the various metabolic reaction of the body but the rate of the formation increased with diabetes mellitus. Protein glycation occurs in the body through non enzymatic glycation reaction in diabetes mellitus and glycation enhances the formation of free radicals. The protein glycation which is the key molecular basis of the diabetic

complications like retinopathy, neuropathy and nephropathy and macro vascular complications like cardiovascular diseases involves several oxidation steps. Antioxidants that prevent or retard the formation of free radicals directly correlate with the preventing diabetic complications.

Freire et al. (1991) found that administration of the crude leaf extract of *S. dulcis* have analgesic and anti inflammatory properties. In the search of bioactive compounds of *S. dulcis*, a β -Glucuronidas inhibitory diterpene called Scoparic A, was isolated together with Scoparic acid B, Scoparic acid C and Scopadulcic acid A, Scopadulcic acid B in the study of Hayashi (2011).

A previous study on the ethanolic extract of *S. dulcis* on the hypoglycemic investigation of the plant extract on blood sugar have reported the highly effective blood glucose lowering activity in alloxan induced hyperglycemic mice (Zulfiker et al., 2010).

Several studies were reported positive results on the antiviral activity, anti-malarial activity, anti cancer activity and neurotropic activity on the *S. dulcis* plant but no information in the literature was reported on antiglycation activity. The whole plant is administrated as a decoction in 120 mL portions twice a day as a treatment of diabetes mellitus in traditional medicine (Ediriweera and Rathnasoriya, 2009). The antidiabetic effect of the plant may be due to the prevention or retard the protein glycation and antioxidant activities.

The efficacy of using the plant as a traditional medicine for diabetes mellitus is due to synergistic effect of antiglycation potential, antioxidant potential and blood glucose lowering effect of different compounds in the plant. Even though this plant is not prescribed in herbal formulations in Ayurveda medicine people use the plant as a traditional medicine for years.

5. Conclusion

The decoction of *Scoparia dulcis* plant prepared according to the traditional method is a good therapeutic potential for diabetes mellitus due to the strong antioxidant potential. Antiglycation potential and high phenolic content.

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