

Hypoglycemic Potentials and Phenolic Characterization of Aqueous Extract of Senna Podocarpa Leaf

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Received: December 4, 2020

Published: February 12, 2021

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Abstract

Diabetes mellitus (DM) is a life threatening metabolic disorder characterized by hyperglycemia. Senna podocarpa is an evergreen glabrous shrub plant whose leaves are used in folklore medicine as poultices for the treatment of wounds, swellings, skin diseases, yawns and diabetes. This study was carried out to determine the effect of Senna podocarpa on carbohydrate hydrolyzing enzymes (α -amylase and α -glucosidase) and to analysis the phenolic profiles of the plant. Enzyme inhibitory assay of α -amylase, α -glucosidase and Fe²⁺-induced lipid peroxidation in rat pancreas were carried out. In addition, DPPH radical scavenging ability, iron chelating capacity, ferric reducing antioxidant property (FRAP), total phenolic content, and total flavonoid content and HPLC-DAD phenolic analysis of Senna podocarpa were investigated. Tukey test at $p < 0.05$ was the statistical tool used. The results revealed that the aqueous extract of Senna podocarpa leaves had inhibitory actions on α -amylase, α -glucosidase and Fe²⁺-induced lipid peroxidation in rat pancreas. The extract also showed DPPH radical scavenging ability and iron chelating capacity in concentration-related fashion and the ferric reducing antioxidant property (0.49 ± 0.01), total phenolic contents (0.96 ± 0.01) and total flavonoid content (0.56 ± 0.01). Furthermore, HPLC-DAD phenolic analysis shown the occurrence of the chlorogenic acid, catechin, caffeic acid, ellagic acid, epicatechin, quercitrin, quercetin and kaempferol and gallic acid. Nevertheless, chlorogenic acid was the most abundant. The results obtained from this study showed that aqueous extract of Senna podocarpa leaf had strong inhibitory action on carbohydrate hydrolyzing enzymes and potent antioxidant property which might be feasible mechanistic approach supporting its use for the treatment of diabetes mellitus. This hypoglycemic action could be due to the preponderance of phenolics and phenolic acids present in this plant.

Keywords: Senna Podocarpa; HPLC-DAD; Diabetes; Phenolic; Antioxidant; Pancreas

Introduction

An essential feature in the cure of type 2 diabetes is the regulation of the surge in post prandial glucose concentrations [1]. Hence, starch digestion delay by enzyme inhibition of carbohydrate-hydrolysing enzymes plays a significant role in the regulation of diabetes. Pancreatic alpha-amylase negative effectors retard the digestion of carbohydrate resulting in a diminution of glucose absorption rate and the post-prandial glucose level reduc-

tion [2]. Many inhibitors presently in medical usage include miglitol and acarbose that impede glycosidases like alpha-glucosidase and alpha amylase but others for example voglibose prevent alpha glucosidase [3].

Conversely, there are disadvantages of several man-made hypoglycemic drugs. This include adverse side reactions, not precise and inability to ameliorate diabetic complications. The actual side effects of these negative effectors are gastric or stomach related

viz., distending, gut uneasiness, diarrhea and gassiness [3]. More attention is now given to medicinal plants for the management of diabetes mellitus because of their little or no side effects and also that they are cheaper and more available in comparison to the conventional hypoglycemic drugs [4,5].

The restrictions and side effects connected with prevailing conventional oral hypoglycemic drugs had required the exploration for novel medicines. Based on this, plant metabolites now serve as substitute object to get nascent antioxidant and antidiabetic remedies owing to their folklore usage.

Patients with diabetes exhibit momentous accentuated levels of oxidative stress which result into major nervous, circulatory, retinal, kidney diabetic problems. Human body produces an array of fortifications against oxidative stress at their distinctive stages. Natural products have been implicated in the reduction of reactive oxygen species synthesis, boosting of endogenous enzymes of antioxidant function such as Superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase, in addition to glutathione reductase (GSH) and DNA repair system. High blood glucose prominently reduces glutathione threshold lowering defenses against stress-induced oxidative damage. Senna podocarpa is a tree that could mature to a height of 5 metres. Senna podocarpa previously called *Cassia podocarpa* guill. (leguminosae - Caesalpinioideae) is a smooth plant of 5 m in height. It is widely utilised in tradomedicine in the cure of skin ailments. The foliage is recognized for its anti-gonorrhoeal and emetic potentials in addition to a guinea worm and painful curative therapy in the midst of Igbos and Yoruba languages of Nigeria, where it is commonly called Agelo-ogala and Asunwon, correspondingly. This study was therefore carried out to determine the effect of Senna podocarpa on carbohydrate hydrolyzing enzymes (α -amylase and α -glucosidase) and to analysis the phenolic profiles of the plant.

Material and Methods

The organic solvents, 1,1-diphenyl-2-picrylhydrazyl (DPPH) in addition to reagents such as ferrozine, ferric chloride, sodium carbonate, potassium ferricyanide, trichloroacetic acid, tris HCl, aluminum nitrate, potassium acetate, butylated hydroxytoluene, gallic acid, Folin's reagent, ascorbic acid and quercetin were utilized. The entire reagents were of pure grade. Methanol, acetic, gallic, chlorogenic, ellagic and caffeic acids were obtained from Merck (Darm-

stadt, Germany). Catechin, Epicatechin, quercetin, quercitrina and kaempferol were acquired through Sigma Chemical Co. (St. Louis, MO, USA). A Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan) fortified with Shimadzu LC-20AT interchanging pumps linked to a DGU 20A5 degasser coupled with a CBM 20A integrator, LC solution and SPD-M20A diode array detector were employed for the High performance liquid chromatography (HPLC-DAD) analysis.

Plant material

Fresh aerial part (twigs) of Senna podocarpa were collected from Akungba Akoko, Ondo State, and were authenticated in the Department of Plant Science and Biotechnology.

Preparation of extract

The leaves of Senna podocarpa were obtained, rinsed and dried at ambient temperature. After grinding into the fine powder, it was extracted leaves was removed from its stalk and washed under running water and then dried. The dried leaves were grounded into fine powder making use of blender. 5g of the powdery form of the plant was weight using analytical weighing balance and dissolved in 100ml of distilled water (using bottle). The bottles were arranged in oscillator or a shaker calibrated at 242r/min and allowed for 3 hours in order to extract all the phytochemicals into the water. After 3hours the oscillator was stopped and the content of the tube was filtered making use of filter paper to separate the filtrate from the residue. The filtrate was kept in separate covered container.

Total phenolic determination

Total phenolics content was measured based on Folin-Ciocalteu method, the standard used was gallic acid. The powdery extract (5g) was mixed in 1 ml 50% of methanol. A mixture of extract (0.5 ml) with 0.5ml of 50% Folin- Ciocalteu reagent was carried out. After few minutes, 1.0 ml of 20% Na_2CO_3 was included to the mixture and incubated for 10 min at ambient temperature. The mixture was spinned using a centrifuge at 150 g for 8 min and the supernatant absorbance was measured at a wavelength of 730 nm. The result was expressed as gallic acid equivalents (GAE) in milligrams per gram sample [6].

Ferric reducing property determination

The reducing power of the extracts was detected by evaluating the capacity of the extracts to reduce FeCl_3 solution was added

by method of Oyaizu [7]. 2.5ml aliquot was mixed with 2.5ml was mixed with 2.5ml 200M sodium phosphate buffer (pH 6.6) and 2.5ml 1% potassium ferricyanide. The incubation of the mixture was done at 50°C for 20mins. Thereafter, 2.5ml of 10% trichloroacetic acid was included. The combination was spun at 650rpm for 10 minutes. The supernatant (5mL) was combined with the same volume of water and 1ml 0.1% ferric chloride. The absorbance measurement was carried out at 700nm using JENWAY UV-Visible spectrophotometer.

Determination of total flavonoids

The total flavonoid content was examined with a little modification of Meda, *et al.* [8]. Concisely, 0.5ml of suitable diluted sample was added with 0.5ml methanol, 50ul 10% AlCl₃, 50ul 1M potassium acetate and 1.4ml water, and left for incubation at room temperature for 30 minutes.

The supernatant absorbance was determined at 415 nm, 40 minutes later at room temperature. Quercetin was the standard used for the calculation.

Antioxidant testing assays

In DPPH assay as reported by Chu, *et al.* [9]. For 1000 µg/ml sample solution, 1 mg of fine extract was liquefied in 1 ml of 50% ethanol solution. Serial dilution of 100, 250, 400, 550, 700, 850µg/ml with 50% ethanol was made for the solution. For a separate reaction, the solutions were combined with 1 ml of 0.1 mM 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 0.45 ml of 50 mM tris-HCl buffer (pH 7.4) and 0.05 ml samples at room temperature for 30min. The negative control consists of 50% of ethanol. The reduction of the DPPH free radical was Where A0 was the absorbance of the control (blank, without extract) and A1 was the absorbance of the extract. The percentage of ferrous ion chelating effect of test samples were evaluated and matched with that of BHT (butylated hydroxytoluene), which serve as the standard. The reducing potential of the extracts was determined employing the method of Oyaizu [7]. Varied concentrations of each extract i.e. 50, 100, 150, 200, 250 and 300 µg ml⁻¹ in methanol, (2.5 ml) of each were added with 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide and the mixture was left for incubation at 50°C for 20 minutes. Aliquots (2.5) ml of 10% trichloroacetic acid (w/v) was introduced to the solution and later spun for 10 min at 1036 g. The higher layer (5 ml) of the solution was mingled with 5 ml

of deionized water plus 1 ml of 0.1% ferric chloride. After shaking the mixture, it was kept in the dark for 10mins for incubation thereafter the absorbance was read at 700nm. BHT (butylated hydroxytoluene) was used as the control and measured by reading the absorbance at 517 nm [10].

$$\% \text{ inhibition} = [(A0 - A1) / A0] \times 100$$

Where A0 was the absorbance of the control (blank, without extract) and A1 was the absorbance of the extract. The antioxidant activity of each sample was expressed in terms of IC50 (micro molar concentration required to inhibit DPPH radical formation by 50%), calculated from the inhibition curve.

Quantification of compounds by HPLC-DAD

Reverse phase chromatographic analyses was conducted under gradient settings employing C18 column (4.6 mm x 150 mm) filled with 5µm diameter particles; the mobile phase was water comprising 2% acetic acid (A) and methanol (B), and the composition gradient was: 5% (B) for 2 min; 25% (B) until 10 min; 40, 50, 60, 70 and 80% (B) every 10 min [11]. Senna podocarpa extract and mobile phase were sieved through 0.45 µm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use, the Senna podocarpa extract was analyzed at a concentration of 15 mg/mL. The flow rate was 0.6 ml/min, injection volume 40 µl and the wavelength were 254 for gallic acid, 280 nm for catechin and epicatechin, 325 nm for caffeic, chlorogenic and ellagic acids, and 365 nm for quercetin, quercitrin and kaempferol. All the samples and mobile phase were sieved through 0.45 µm membrane filter (Millipore) and degassed by ultrasonic bath before use. Solutions of stock for standards were produced in the HPLC mobile phase at a concentration range of 0.025 – 0.300 mg/ml for catechin, epicatechin, kaempferol, quercetin and quercitrin; and 0.010 – 0.250 mg/ml for gallic, chlorogenic, ellagic and caffeic acids. Chromatography peaks were established by matching its retention time with those of reference standards and by DAD spectra (200 to 600 nm). Calibration curve for gallic acid: $Y = 12563x + 1196.2$ ($r = 0.9995$); catechin: $Y = 13074x + 1267.5$ ($r = 0.9996$); chlorogenic acid: $Y = 12781x + 1267.2$ ($r = 0.9997$); caffeic acid: $Y = 12648x + 10976.3$ ($r = 0.9992$); ellagic acid: $Y = 13279x + 1187.3$ ($r = 0.9998$); epicatechin: $Y = 13274x + 1186.3$ ($r = 0.9994$); quercitrin: $Y = 12465x + 1305.7$ ($r = 0.9999$); quercetin: $Y = 11965x + 1262.5$ ($r = 0.9999$) and kaempferol: $Y = 12539x + 1358.7$ ($r = 0.9995$). All chromatog-

raphy processes were performed at room temperature and three replicates.

The limit of detection (LOD) and limit of quantification (LOQ) were considered established on the standard deviation of the responses and the slope using three independent analytical curves. LOD and LOQ were calculated as 3.3 and $10 \sigma/S$, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve [11].

DPPH radical scavenging activity of various extracts of Senna podocarpa leaves and ascorbic acid are shown in Figure 1. In this study, it was detected that varied extracts of Senna podocarpa mopped up DPPH free radical in a dose dependent form because, as the concentration of extracts increased the DPPH scavenging activity also improved. The direction of effectiveness of the extracts was $(10.4\%) > (23.7\%) > (43.8\%)$ respectively at maximum concentration. The DPPH scavenging ability of aqueous solution of leaf is presented in figure 1. The extract showed high scavenging ability $(16.7-51.2\%)$ in a concentration dependent pattern $(0.0042-0.0167\text{g/ml})$. The Lipid peroxidation assay result is depicted in figure 2. A decreasing trend of the percentage MDA produced was noted as the concentration rises. The extract produced low MDA $(75\%-33.3\%)$ from $(0.001-0.004)$ in a concentration linked fashion. The iron chelating capacity of the extract is shown in figure 3. The extract exhibited high chelating ability $(10.4-35.8\%)$ from $(0.001-0.004\text{g/ml})$ in a concentration reliant form. The interaction of the extract of Senna podocarpa with α -amylase is represented in figure 4. The extract displayed a high level of inhibition $(33.3-84.6\%)$ in a concentration dependent manner from $(0.001-0.004\text{g/ml})$. The interaction of the extract with α -glucosidase is presented in figure 5. The extract showed a high level of inhibition $(14.3-64.3\%)$ from $(0.001-0.004\text{g/ml})$ in a concentration related pattern. The results of the total phenol content, total flavonoid content, and FRAP of the aqueous extract of Senna podocarpa were $0.96 \pm 0.01\text{mg/mL}$, $0.56 \pm 0.01\text{mg/mL}$ and $0.49 \pm 0.01\text{mg/mL}$ respectively. HPLC fingerprinting of Senna podocarpa aqueous extract showed the presence of the gallic acid ($t_R = 10.17$ min; peak 1), catechin ($t_R = 16.35$ min; peak 2), chlorogenic acid ($t_R = 21.05$ min; peak 3), caffeic acid ($t_R = 25.92$; peak 4), ellagic acid ($t_R = 32.47$ min; peak 5), epicatechin ($t_R = 37.19$ min; peak 6), quercitrin ($t_R = 45.19$ min; peak 7), quercetin ($t_R = 49.71$ min; peak 8) and kaempferol ($t_R = 60.14$ min; peak 9) (Figure 6 and Table 1). From the HPLC-DAD fingerprinting result, it

was observed that chlorogenic acid ($51.65 \pm 0.01\text{mg/g}$) is the most abundant phenolic acid in Senna podocarpa followed by caffeic acid ($40.52 \pm 0.02 \text{ mg/g}$) while quercitrin ($40.31 \pm 0.02 \text{ mg/g}$) is the most pronounced flavonoid.

Figure 1: DPPH Scavenging ability of aqueous extracts of Senna podocarpa.

Figure 2: Lipid peroxidation of aqueous extracts of Senna podocarpa.

Figure 3: Iron chelating ability of aqueous extracts of Senna podocarpa.

Figure 4: α -amylase inhibitory activity of Senna podocarpa.

Figure 5: α -glucosidase inhibitory activity of Senna podocarpa.

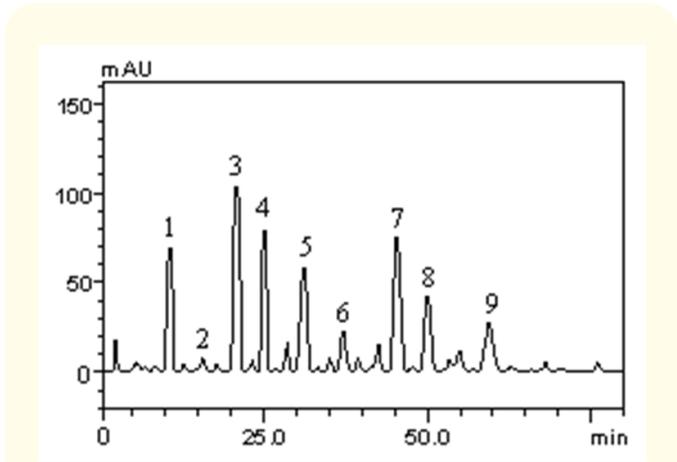


Figure 6: Representative high performance liquid chromatography profile of Senna podocarpa leaves extract. Gallic acid (peak 1), catechin (peak 2), chlorogenic acid (peak 3), caffeic acid (peak 4), ellagic acid (peak 5), epicatechin (peak 6), quercitrin (peak 7), quercetin (peak 8) and kaempferol (peak 8).

Compounds	Senna podocarpa			
	Extract		LOD	LOQ
	mg/g	%	$\mu\text{g/mL}$	$\mu\text{g/mL}$
Gallic acid	36.90 \pm 0.03 a	3.69	0.009	0.028
Catechin	2.76 \pm 0.01 b	0.27	0.035	0.116
Chlorogenic acid	51.65 \pm 0.01 c	5.18	0.013	0.042
Caffeic acid	40.52 \pm 0.02 d	4.05	0.024	0.081
Ellagic acid	31.20 \pm 0.03 e	3.12	0.019	0.063
Epicatechin	13.78 \pm 0.02 f	1.37	0.012	0.039
Quercitrin	40.31 \pm 0.02 d	4.03	0.015	0.049
Quercetin	25.16 \pm 0.01 g	2.51	0.028	0.092
Kaempferol	18.35 \pm 0.03 f	1.83	0.017	0.056

Table 1: Phenolics and flavonoids composition of Senna podocarpa.

Results are expressed as mean \pm standard deviations (SD) of three determinations. Averages followed by different letters differ by Tukey test at $p < 0.05$.

Discussion

DPPH is a stable radical that has been extensively used to assess the antioxidant activity of numerous natural products [12]. The concentration-dependent scavenging ability of the aqueous extract of this plant was similar to the report of Oboh, *et al.* [13] which showed that cooked clovebud scavenged the free radical generated by DPPH in the same manner as Senna podocarpa. This posited the antioxidant capacity of this plant. The relative Malondialdehyde (MDA) produced of extracts of Senna podocarpa express marked variations. Inhibition of lipid peroxidation of the pancreas as demonstrated by the leaf extract also gave a credence to the possibility of quenching the free radical produced. It is important to stress the negative impact of free radicals in the cause of diabetes. The ability of this plant to reduce peroxidation of pancreatic lipid may play a major role in the management of diabetes mellitus. This is akin to the report of Kooti, *et al.* [14].

There is correlation between reducing properties and reductone presence [15] which have been observed to elicit antioxidant potential by disrupting the free radical chain with a hydrogen atom donation [16]. Prevention of peroxide formation by reductones have also been reported.

Ferrozine can quantitatively form complexes with Fe²⁺. Availability of chelating agents leads to the disturbance of complex creation, causing decline of red colored composite. Amount of colour reduction is the measurement of metal chelating capacity. As shown in Figure 2, aqueous extracts of Senna podocarpa along with standard compound impede with the development of ferrous and ferrozine complex signifying that they have chelating activity and are capable of arresting the ferrous ions before the creation of ferrozine. The probable elucidation of chelating power of the extracts is the capacity of the extracts to reduce iron and then to produce Fe²⁺ extracts complex that are passive. This research is in concordance with comment made in some literature that non-flavanoid polyphenolics can reduce iron and the generate Fe²⁺- polyphenol complexes that are inactive [17]. The antioxidant capacity of phenolic compounds is significantly as a result of their redox properties, that could portend a critical role in absorption and free radical quenching, stopping singlet and triplet oxygen, or decomposition of peroxides [18]. In order to measure the ability of extract to reduce iron, it has been discovered that the Fe³⁺- Fe²⁺ alteration occurred in the company of extract samples which had been hypothesized formerly by Oyaizu [7].

In this study, aqueous extract of this plant inhibited the carbohydrate hydrolyzing enzymes. This result is similar to previous studies which revealed that quercetin, rutin etc, flavonoids inhibited α -glucosidase and α -amylase by showing hypoglycemic potentials in experimental animals [19,20]. Inhibition of the activities of α -glucosidase and α -amylase will result in the decrease in the post-prandial hyperglycemia [21].

From the HPLC-DAD fingerprinting it was observed that chlorogenic acid is the most abundant phenolic acid in Senna podocarpa followed by caffeic acid while quercetrin is the most pronounced flavonoid. Chlorogenic and caffeic acid have been shown have strong antioxidant properties and also demonstrated inhibitory action on carbohydrate-hydrolysing enzymes [10].

Conclusion

The results revealed that the aqueous extracts of Senna podocarpa leaves has higher reducing power, inhibited lipid peroxidation by reducing the MDA production, showed high DPPH radical scavenging ability and inhibited α -amylase and α -glucosidase in dose dependent pattern. This result revealed the extracts has high

antioxidative properties and inhibitory action on carbohydrate hydrolyzing enzymes which could be part of the mechanism by which the plant is used in the treatment of diabetes and provide a justification for the therapeutic use of the plants. The present data suggested that the extract of this plant could be a potential source of natural antidiabetic therapy and antioxidant that may be of valuable importance for the treatment of diseases. Further studies need to identify other phytochemicals vis-à-vis mechanism of action and to establish their pharmacological properties using appropriate assays model.

Significant Statement

This study discover that aqueous extract of Senna podocarpa leaf that can be beneficial for the management of diabetes mellitus. This study will aid the researchers to discover the critical areas of usage of medicinal plants which are cheaper, no or less side effects that many researchers were not able to explore. Thus a new theory on alternative and complementary medicine may be advanced.

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