INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disorder that stems from the dysregulation of carbohydrate, protein, and lipid metabolism. It is characterized by an increased blood glucose level (hyperglycaemia) due to the inefficiency or deficiency of insulin [1]. Prolonged hyperglycaemia has been established as the primary cause of diabetes-related complications and the formation of reactive oxygen species (ROS) [2,3]. At high concentrations, ROS can lead to oxidative-stress-mediated organ damage and DNA damage [2]. They can also contribute to the progression of cancer, diabetes, cardiovascular disease, Alzheimer’s, and other degenerative diseases [4].

Presently, diabetes remains in the top five causes of death in the Caribbean. According to the International Diabetes Federation (IDF), diabetes affects 9% of the global adult population, most of whom reside in low to middle-income countries. In Jamaica, 11.7% of the adult population (20-79 years) and was the primary cause of death and disability-affected-years [5,6]. The current management of Type 2 diabetes includes drug therapy that is aimed at reducing hyperglycaemia. However, the medicines available have reduced user compliance rates, typically due to their cost and undesirable side-effects [7]. The use of alternative therapy, primarily plant-based remedies (folk/traditional medicine), continues to provide primary health care in developing countries [8]. The increased use of folk medicine versus conventional medicine is particularly evident in rural populations where the practice of folk medicine is frequent [8]. Plants are an established source of bioactive compounds (phytochemicals) that can provide a host of therapeutic benefits as anti-microbial, antidiabetic, and antioxidant agents [9]. The use of various phytochemicals continues to influence pharmaceutical drug development due to their contribution to human health and reduced toxicity. Common phytochemicals found to possess therapeutic effects include alkaloids, terpenoids and polyphenols, which have formed the basis for several therapeutic agents used worldwide [10].

Correspondence to: Ruby L. Alexander-Lindo, Department of Basic Medical Sciences, Faculty of Medical Sciences and Technology, The University of the West Indies (UWI), Mona, Kingston 7, Jamaica; Tel: +18763357924; Fax: +18769777852; E-mail: lisa.lindo@uwimona.edu.jm

Received: September 12, 2020, Accepted: November 07, 2020, Published: November 13, 2020


Copyright: © 2020 Peddie DA, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Smilax balbisiana (Chainy Root) is a member of the Smilax genus, which is a diverse group of shrubs belonging to the Liliaceae family. It is endemic to Jamaica and is popularly used in “Roots” tonic as an aphrodisiac to treat impotence, increase strength, and purify the blood [11,12]. The rhizomes of these plants are described as “thick, elongated, and tuberous.” They are the core parts used for their ethnomedicinal properties, as noted by herbalists in the Marron village of Accompong, Jamaica [12]. Other traditional uses include the treatment of anaemia, syphils, diabetes, and hypertension [13]. Several species from the Smilax genus are popularly used in Traditional Chinese Medicine for the treatment of similar ailments, including syphils, rheumatoid arthritis, inflammatory diseases, and skin diseases [14]. In other areas, Smilax species have been reported to possess antidiabetic, hypotensive, anti-inflammatory, and other biological properties [15-19].

To the best of our knowledge, no published data have been found regarding the phytochemical constituents or biological activities of S. balbisiana. With this framework, the present study investigated the use of this plant for its hypoglycaemic effect in Sprague-Dawley rats.

**MATERIALS AND METHODS**

**Plant material and extract preparation**

The University of the West Indies, Mona Campus Research Ethics Committee approved this study in 2015 (Reference No. AN, 8, 14/15). Fresh rhizomes of Smilax balbisiana (Chainy Root) were collected during March and December 2016 from Bethsalem, St. Elizabeth, Jamaica. A specimen was authenticated by Dr Ina Vandebroek (from the New York Botanical Garden) and Mr Patrick Lewis at The University of the West Indies (UWI), Mona Herbarium. A deposit was made and assigned the Voucher number 3633.

Fresh samples of Chainy Root rhizomes were washed to remove extraneous materials and airdried for five days (shaded from direct sunlight; 30°C). The rhizomes (570 g) were milled to a fine powder, weighed and extracted with hexane, ethyl acetate and later, methanol. Solvent extraction involved saturating the powder with fresh hexane for two separate eight (8) hour periods and one twenty-four-hour (24) period. The filtrates were collected and concentrated at 60°C using rotary evaporation to obtain the crude hexane extract (1.3 g, 0.23% yield). The procedure was repeated using ethyl acetate (5.3 g; 0.93% yield) then later, methanol (47.4 g, 8.32% yield). The extracts were stored at -20°C for further studies.

**Phytochemical screening of S. balbisiana crude extracts**

The crude extracts of Smilax balbisiana rhizome were subjected to phytochemical screening using standard methods [20,21]. Dragendorff’s and Hager’s reagents were used to detect the presence of alkaloids while the stain test was used to identify fixed oils. The lead acetate test and alkaline reagent test were used to detect flavonoids. Liebermann-Burchard test and Salkowski’s test were used to detect phytoesters. Benedict’s and Fehling’s reagents were used for reducing sugars. Foam and Froth tests were used to identify saponins and ferric chloride solution for the detection of tannins.

**Hypoglycaemic activity (Acute experimental model)**

**Animals**

Healthy Sprague-Dawley (S-D) rats (46 weeks old; 150-200 g) of equally mixed sexes were obtained from the UWI Mona, Animal House. The rats were divided into groups of six rats each. Before experimentation, they were weighed then fasted overnight (approximately 12 hours) with free access to water.

**Oral glucose tolerance test using S. balbisiana crude extracts**

An oral glucose tolerance test (OGTT) was performed using a modification of an established protocol [22]. Following an overnight fast, an incision was made at the tip of the rats’ tails, and a fasting blood glucose sample was measured. Immediately after, the crude extract (200 and 300 mg/kg BW), glibenclamide (positive control; 5 mg/kg BW) or carrier (vehicle; negative control) was administered orally. Blood glucose levels were measured for 1 hour at 30-minute intervals, post-administration. After this, a glucose load of 1.75 g/kg BW was administered orally using a gavage needle. Blood glucose readings were taken at 30-minute intervals for a further two and a half hours.

The ACCU-Chek Active Blood Glucose Monitoring System was used to measure blood glucose concentrations. Corn oil or DMSO was used as the carrier (vehicle).

2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) free radical scavenging assay

A modification of a 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay method [23] was used to assess the antioxidant activity of the ethyl acetate and methanol crude extracts from S. balbisiana rhizome. In brief, a solution of DPPH (0.1 mM) was prepared in methanol. Each crude extract was prepared in methanol at varying concentrations (0.08 to 1000 µg/mL). Each reaction mixture consisted of 2 mL of DPPH, which was added to 2 mL of each extract. The mixture was agitated and incubated in the dark at room temperature for 30 minutes. The decrease in DPPH radicals was measured using a spectrophotometer at a wavelength of 517 nm. The reference standard, ascorbic acid (positive control), was prepared similarly at the concentrations mentioned above and assayed in triplicates along with the extracts. A decreased absorbance reading indicated a higher free radical scavenging capacity. Percentage DPPH inhibition was calculated using the formula:

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

The results were reported as IC\textsubscript{50}. The IC\textsubscript{50} value represents the half-maximal inhibitory concentration that resulted in 50% inhibition of DPPH radicals.

**Statistical analysis**

All data were presented as mean ± Standard Error of the Mean (SEM). Statistical analysis was carried out using a statistical package software (SPSS, Version 22, Chicago, IL, USA) to perform the Student’s t-test and Tukey-Kramer multiple range post-hoc test. Variations between the groups where \(p \leq 0.05\) was considered significant.

**RESULTS AND DISCUSSION**

The hypoglycaemic effect of the crude extracts from S. balbisiana...
Peddie DA, et al.

rhizomes was studied in Sprague-Dawley (S-D) rats using the OGTT. Oral administration of the hexane and ethyl acetate crude extract (200 mg/kg BW) did not produce any significant change in blood glucose levels when compared with their respective controls (p>0.05; Figures 1 and 2, respectively). However, oral administration of the methanol extract (200 mg/kg BW) resulted in a significant increase in blood glucose level at the 60-minute interval (5.68 ± 0.24 mmol/L vs. 4.78 ± 0.25 mmol/L; p=0.025), as well as from the 90-minute to the 120-minute intervals (p ≤ 0.05) when compared with the control respectively (Figure 3). This increase may have been due to the abundance of reducing sugars, which are concentrated in the polar methanol extract, as indicated in the phytochemical analysis (Table 1). In contrast, an increase in the dosage of the methanol extract to 300 mg/kg BW produced significant hypoglycaemia at the 120-minute interval (4.84 ± 0.50 mmol/L vs. 6.59 ± 0.32 mmol/L; p=0.014) and 150-minute interval (5.05 ± 0.19 mmol/L vs. 6.01 ± 0.32 mmol/L; p=0.03) in comparison with its control, dimethyl sulfoxide (DMSO; Figure 3). The increased dosage of the methanol extract would have allowed the active compounds to reach sufficient levels to mitigate the effect of the high sugar content as observed (Figure 3).

Figure 1: Effect of Smilax balbisiana rhizome hexane crude extract on blood glucose concentration. Dose at 200 mg/kg B.W. ( ) and 300 mg/kg B.W. ( ) with glibenclamide at 5 mg/kg B.W. ( ) versus corn oil control ( ) via oral administration in S-D rats. Data expressed as mean ± SEM (n=6). * = p≤ 0.05 vs. control. ** = p≤ 0.01, multiple range post-hoc test.

Figure 2: Effect of Smilax balbisiana rhizome ethyl acetate crude extract on blood glucose concentration. Dose at 200 mg/kg B.W. ( ) and 300 mg/kg B.W. ( ) versus DMSO control ( ) control via oral administration in S-D rats. Data expressed as mean ± SEM (n=6). * = p≤ 0.05 vs. control.

The increased dose of the hexane extract to 300 mg/kg BW was able to produce the greatest hypoglycaemic response, following an oral administration of glucose. A significant reduction was observed at the 90-minute interval (5.85 ± 0.37 mmol/L vs. 6.92 ± 0.22 mmol/L; p=0.033) and 120-minute interval (5.71 ± 0.16 mmol/L vs. 6.59 ± 0.32 mmol/L; p=0.034) respectively (Figure 2).

The increased dosage of the hexane extract to 300 mg/kg BW was able to produce the greatest hypoglycaemic response, following an oral administration of glucose. A significant reduction was observed at the 90-minute interval (5.08 ± 0.24 mmol/L vs. 6.06 ± 0.16 mmol/L; p=0.007) and the 120-minute interval (4.93 ± 0.24 mmol/L vs. 6.22 ± 0.23 mmol/L; p=0.003) when compared with its control, corn oil (Figure 1). The hexane extract was compared with the known oral hypoglycaemic agent, glibenclamide (5 mg/kg BW). Glibenclamide produced significant hypoglycaemia throughout the glucose tolerance test when compared with both the hexane crude extract and the control (p ≤ 0.01; Figure 1). Glibenclamide is known to stimulate insulin release from the pancreatic β-cells, which leads to its blood-glucose-lowering effect [24]. This hypoglycaemic effect was very pronounced in the normal healthy rats used in this study. The variation in blood glucose levels observed between the hexane crude extract and glibenclamide may be due to the presence of numerous compounds in the crude extract. The myriad of compounds in these crude extracts could have masked the effect of the hypoglycaemic compound(s) present. As seen in these three crude extracts, it is possible that at a lower dosage, the other compounds might have nullified the activity of the active compound(s) or the activity of the hypoglycaemic compound(s) might be present in negligible quantity. However, as the concentration was increased the activity of the active compound(s) was present in sufficient concentration to exert the hypoglycaemic effect observed [25].

Preliminary phytochemical analysis of Smilax balbisiana rhizomes detected an abundance of alkaloids, fixed oils, phytosterols, and terpenoids in the hexane crude extract. A moderate amount of all phytoconstituents tested were present in the ethyl acetate crude extract. The similarity in the amount of phytochemicals detected could have indicated why the hypoglycaemic effect of the ethyl acetate extract was not as notable as that displayed by the hexane extract. In contrast, the methanol extract contained a moderate concentration of flavonoids, fixed oil, tannins, terpenoids, and phytosterols, along with an abundance of reducing sugars and

Figure 3: Effect of Smilax balbisiana rhizome methanol crude extract on blood glucose concentration. Dose at 200 mg/kg B.W. ( ) and 300 mg/kg B.W. ( ) with DMSO control ( ) control via oral administration in S-D rats. Data expressed as mean ± SEM (n = 6). *=p ≤ 0.05 vs. control.

Med Aromat Plants (Los Angeles), Vol. 9 Iss. 6 No: 364
In general, an increased dosage of the crude extracts resulted in significant hypoglycaemic responses when compared with their respective controls. These responses indicated that a dose-dependent effect was associated with the hypoglycaemic activity of *S. balbisiana* rhizome extracts. Crude plant extracts are known to contain an unspecified number of phytochemicals. The concentration of these compounds may influence the action of the bioactive compound(s) present. It was observed that the crude extract had the potential to reduce blood glucose levels significantly. Therefore, these hypoglycaemic responses would become more pronounced for the isolated bioactive compound(s).

The hexane crude extract contained a high concentration of phytosterols/terpenoids and alkaloids. Phytosterols are known to stimulate insulin secretion [28]. The ethyl acetate extract (IC$_{50}$=32.48 µg/mL) had a more substantial antioxidant effect than methanol extract (IC$_{50}$=58.36 µg/mL) and was comparable to the positive control, ascorbic acid (IC$_{50}$=15.88 µg/mL). Similar results were reported for *S. chinensis* L, where the ethyl acetate extract produced a more potent antioxidant effect compared with the methanol extract [34,35]. The antioxidant effect was reported to contribute to the significant hepatoprotective effect of the *S. chinensis* L [33]. *S. larrvata* ethyl acetate extract also produced moderate antioxidant activity compared to ascorbic acid. It was linked to the presence of various flavonoids, including kaempferol which is found throughout the *Smilax* genus [35]. The hydrogen donating ability of the ethyl acetate extract can be linked to the presence of ubiquitous phytochemicals, including flavonoids, tannins, and saponins, which have established antioxidant properties [36]. These compounds may, therefore, protect against hyperglycaemia-induced oxidative stress that contributes to the development of diabetic complications [37].

### Table 1: Phytochemical constituents of *Smilax balbisiana* rhizome extracts.

<table>
<thead>
<tr>
<th>Phyto-constituents</th>
<th>Test (s)</th>
<th>Hexane extract</th>
<th>Ethyl acetate extract</th>
<th>Methanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Drenganford’s test</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Hager’s test</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Fixed oils</td>
<td>Stain test</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Lead acetate test</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Alkaline reagent test</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phytosterol and terpenoids</td>
<td>Lieberman-Burchard’s test</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Salkowski’s test</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Reducing sugars Carbohydrates</td>
<td>Benedict’s test</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Fehling’s test</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Saponins</td>
<td>Foam test</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Froth test</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Tannins</td>
<td>Ferric chloride test</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Keys**: (++ = Abundant; (+) = Moderate; (-) = Absent.

### Table 2: 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) free radical scavenging effect of various crude extracts on *Smilax balbisiana*.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Free Radical Scavenging Activity (%)$^a$</th>
<th>IC$_{50}$ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl acetate</td>
<td>89.34 ± 0.00</td>
<td>32.48</td>
</tr>
<tr>
<td>Methanol</td>
<td>83.66 ± 0.33</td>
<td>58.36</td>
</tr>
<tr>
<td>Ascorbic acid (positive control)</td>
<td>94.42 ± 0.33</td>
<td>15.88</td>
</tr>
</tbody>
</table>

$^a$ -Inhibitory activity at the maximum concentration (µg/mL)

Saponins (Table 1). In particular, saponins have been reported to exert hypoglycaemic effect via numerous mechanisms, including the promotion of insulin release from the beta-cell islets, activation of glycogen synthesis, inhibition of gluconeogenesis, inhibition of αglucosidase, and inhibition of mRNA expression of glycogen phosphorylase and glucose 6-phosphatase [26]. Furthermore, several of these classes of phytochemicals have been linked to the hypoglycaemic and antidiabetic effect of *Smilax china* L. rhizome [27]. The mechanism by which these phytochemicals produce hypoglycaemia mainly involves an increase in pancreatic glucose uptake, the inhibition of intestinal alpha-glucosidase activity, or the stimulation of insulin secretion [28].
CONCLUSION

Oral administration of the crude extracts of Smilax balbisiana rhizome indicated a dose-dependent hypoglycaemic effect. The hexane extract produced a significant glycaemic lowering of all three extracts in Sprague-Dawley rats. The hypoglycaemic effect of the hexane crude extract was attributed to the presence of alkaloids, phytosterols, and terpenoids, which were detected at high concentrations. The antioxidant assay indicated that the ethyl acetate crude extract, which contained a moderate concentration of polyphenols and flavonoids, produced potent free radical scavenging effect against DPPH free radicals. This study provides beneficial insight into the traditional use of Smilax balbisiana (Chainy Root) rhizome in the management of hyperglycaemia as well as its potential antioxidant effect against hyperglycaemic-induced oxidative stress associated with diabetes. The ethnopharmacological properties of S. balbisiana extracts can lead to its integration as an adjunct or alternative therapy in diabetes management.

ACKNOWLEDGEMENT

The authors acknowledge the Office of Graduate Studies and Research, The University of the West Indies, Mona, for their financial support. The authors also thank Mr Patrick Lewis for authenticating the plant.

AUTHOR CONTRIBUTIONS

D. A. Peddie - Performed data collection, analysis, and interpretation of data; wrote the manuscript.
S. J. Bryan - Provided critical revision of manuscript and interpretation of data.
C. S. Bowen-Forbes - Provided critical revision of manuscript and interpretation of data.
R. L. Alexander-Lindo - Conceptualized and designed study; assisted with the preparation of the manuscript and corresponding author.

CONFLICT OF INTEREST

All authors have contributed to and approved the final manuscript. We declare no conflict of interest in the publication of this manuscript.

REFERENCES

5. https://www.diabetesatlas.org


