The inhibitory effect of an ethanol extract of *Sida rhombifolia* leaves on key carbohydrate hydrolyzing enzymes

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**ABSTRACT**

**Aim:** The study was conducted to screen the ethanol extract of *Sida rhombifolia* (ESR) leaves for its phytochemical constituents and elucidate some of its possible mechanism of action in lowering hyperglycemia.

**Methodology:** The ethanol ESR was prepared and its effect on carbohydrate hydrolyzing enzymes (α-amylase and α-glucosidase) both *in vitro* and *in vivo*, and glucose uptake by muscle tissues was investigated. Qualitative phytochemical screening was also conducted.

**Results:** *Sida rhombifolia* extract contained bioactive phytochemicals and showed significant dose-dependent inhibition of α-amylase and α-glucosidase with IC$_{50}$ values of 831.76 and 1202.3 µg/ml, respectively. It significantly promoted glucose uptake by rat hemidiaphragms and reduced postprandial glycemia in normal rats administered with starch and sucrose.

**Conclusion:** *Sida rhombifolia* ethanolic extract contains bioactive compounds and displayed strong anti-diabetic properties, therefore, it has potential use as an anti-diabetic agent.

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**KEYWORDS**
Diabetes; Sida rhombifolia; α-amylase; α-glucosidase.

**Introduction**

Diabetes mellitus (DM) is a complex metabolic disorder resulting from either impaired synthesis and secretion of insulin by beta cells of the Islets of Langerhans (type 1 DM) or impaired sensitivity of tissues to insulin action (type 2 DM). It is characterized by chronic hyperglycemia that results in diabetic complications such as retinopathy, neuropathy, and nephropathy because of oxidative stress. Oxidative stress is highly increased in the diabetic state because hyperglycemia promotes the generation of free radicals and weakens the ability of the body's natural anti-oxidation defense systems [1,2]. Free radicals have been extensively implicated in the pathogenesis of DM and its associated macro- and micro-vascular complications. These free radicals are produced due to hyperglycemia, lipid peroxidation [3], and elevated concentrations of heavy metals like arsenic which can be found in agricultural produce like vegetables [4]. The clinical management of DM is based on oral anti-hyperglycemic drugs and exogenous insulin. However, despite the availability of the various medications for the management of DM, its global morbidity, mortality, and prevalence is increasing with projections of 366 million cases by 2030 [5]. α-amylase and α-glucosidase inhibitors are a class of promising drugs for management of postprandial hyperglycemia in type 2 DM. However, they are associated with side effects like hypoglycemia, diarrhea, and abdominal pains. Therefore, there is an urgent need for the discovery of drugs which can manage DM with less or no side effects. In the search for such drugs, botanicals have become of more interest due to their multi-pronged effects on the disease. It has been established that some botanicals possess bioactive phytochemicals like phenols, flavonoids, tannins, saponins, and glycosides which confer various mechanisms of action in managing DM [6].

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In the present study, the ethanol extract of *Sida rhombifolia* (ESR) leaves was evaluated for its anti-diabetic properties. *Sida rhombifolia* belongs to the Malvaceae family and is widely distributed across tropical Africa. The plant is characterized by dark green, diamond-shaped leaves with grayish hairs and spiny stipules on the bases of petioles [7]. The plant is used traditionally for the management of a headache, rheumatism, diabetes, and cardiovascular diseases [8]. Previous studies have demonstrated its free radical scavenging ability, hypoglycemic, and hypolipidemic effects on diabetic animals [9,10]. Therefore, this study was conducted to determine the phytochemicals in ESR and to evaluate its *in vitro* and *in vivo* effects of α-amylase and α-glucosidase.

**Materials and Methods**

**Chemicals and reagents**

Pancreatic α-amylase from porcine, dinitrosalicylic acid (DNSA), and soluble starch were purchased from Sigma Aldrich (USA). Glucose oxidase kit was purchased from Aggape Diagnostics, India. All other chemicals and reagents used were of analytical grade.

**Preparation of plant extract**

The plant was collected in Gaborone along the Notwane river on October 2016. The plant was authenticated at the University of Botswana Herbarium and given voucher specimen number UB 019. The leaves were dried at room temperature and ground to fine powder using a laboratory grinder (Brand name: Zhong Xing, Model number: FW80). ESR was prepared by macerating 100 g of the powdered leaves in 70% ethanol for 32 hours. The mixture was filtered using a Whatman 0.45 µm filter paper and the filtrate was evaporated in vacuum via rotor evaporator. The crude extract was dried at room temperature in a fume hood.

**Qualitative phytochemical analysis**

ESR was screened for bioactive phytochemicals using qualitative methods [11,12].

**In vitro analysis of anti-diabetic properties**

**Measurement of α-amylase inhibitory effects of plant extract/ESR**

To determine the effect of the ESR on α-amylase, a method described by Kamtekar et al. [13] was followed with modifications. Briefly, 0.5 ml of distilled water dissolved plant extract (concentrations 200, 400, 600, 800, and 1,000 µg/ml) was incubated with 0.5 ml of porcine pancreatic α-amylase solution (2 units/ml) in 0.02 M sodium phosphate buffer pH 6.9 with 6.7 mM sodium chloride at 37°C for 10 minutes. Then, 0.5 ml of 1 % starch solution was added and the mixture was further incubated at 37°C for 10 minutes. After incubation, the reaction was stopped by the addition of 1 ml of DNSA reagent and further incubation at 85°C in a water bath for 5 minutes. After 5 minutes, reaction mixture color changed to orange-red and was removed from the water bath and cooled to room temperature. The mixtures were diluted to 5 ml using distilled water and absorbance measured at 540 nm using a Shimadzu UV-Vis spectrophotometer. Control samples were prepared in a similar way except that for each plant extract concentration, the enzyme solution was replaced by a buffer. The experiment was performed in triplicates and α-amylase inhibitory activity was calculated using the following formula:

\[
\text{% inhibition} = \frac{\text{Abs (control)} - \text{Abs (sample)}}{\text{Abs (control)}} \times 100\%.
\]

A plot of percentage inhibition against the logarithm of sample concentration was constructed and the concentration inhibiting 50% (IC$_{50}$) was determined.

**Measurement of mode of α-amylase inhibition by ESR**

To study the mode of inhibition of α-amylase by ESR, different concentrations of starch (substrate) (0.05, 0.1, 0.15, 0.20, and 0.25 M) were used. They were incubated with α-amylase in the absence of ESR (inhibitor) and with 800 µg/ml ESR at 37°C. The amount of glucose released was quantified using a glucose standard curve. The type of inhibition was determined from the constructed Lineweaver–Burk plot based on $K_m$ and $V_{max}$ values.

**Measurement of α-glucosidase (sucrose) inhibitory effects of ESR**

For α-glucosidase inhibition assay, the enzyme was isolated from the small intestines of normal Sprague Dawley rats [14]. Normal male rats weighing 150–200 g were sacrificed under diethyl ether anesthetic and dissected. Small intestines were removed and cleaned with cold normal saline. The luminal surface of the intestines was scrapped out using a microscope slide and the epithelial layer collected and homogenized in phosphate buffered saline pH 7.4 containing 1% Triton ×100 and centrifuged at 12,000 rpm for
The antidiabetic properties of Sida rhombifolia

15 minutes. The pellet was further homogenized in the same buffer with cold butanol added to remove Triton. The sample was partially purified overnight by dialysis method. The protein concentration was estimated by the Lowry method [15] and the sample stored at −20°C until needed for use.

To determine the effect of ESR on sucrose, ESR was diluted to make concentrations; 19.53125–2,500 µg/ml in distilled water. 0.5 ml of ESR solution was incubated with 0.5 ml of sucrose (substrate) solution contained in 50 ml test tubes [16]. The tubes were incubated for 3 minutes at 37°C and after which 0.25 ml of 5 mg/ml crude rat intestinal α-glucosidase was added. After thoroughly mixing the contents, the tubes were at 37°C for 15 minutes. The activity of sucrose was stopped by the addition of 0.5 ml of 2.0 M Tris-HCL buffer (pH 6.9). The amount of glucose liberated was determined using the glucose oxidase kit (Agappe Diagnostics, India) and the percentage inhibition of the enzyme by ESR was calculated from the following equation:

\[ \text{%inhibition} = \frac{\text{Abs (control)} - \text{Abs (sample)}}{\text{Abs (control)}} \times 100\% \]

The experiments were carried out in triplicates and results represented as a mean and standard error of means (SEM). A plot of percentage inhibition against the concentration of ESR was used to determine the inhibitory concentration giving 50% inhibition (IC₅₀).

Measurement of mode of α-glucosidase inhibition by ESR

To determine the type of inhibition exhibited by ESR on α-glucosidase, different concentrations of sucrose (substrate) (0.05, 0.1, 0.15, 0.20, and 0.25 M) were incubated with α-glucosidase in the absence of ESR (inhibitor) and with 1.25 mg/ml ESR at 37°C. A Lineweaver–Burk plot was constructed and \( K_m \) and \( V_{\text{max}} \) values were determined from it.

Effect of ESR on glucose absorption by rat hemidiaphragms

The effect of ESR on glucose uptake by rat hemidiaphragms was conducted based on a method described by Ahmed and Urooj [17] with minor changes. Fresh diaphragms were harvested from overnight fasted normal Sprague Dawley rats sacrificed under diethyl ether. The diaphragms were divided into two equal halves, rinsed with normal saline, and placed in well-labeled test tubes containing different media as per the following groups;

**Normal control (NC):** 2 ml of Tyrode solution with 2% glucose and 2 ml of distilled water.

**Positive control (PC):** 2 ml of Tyrode solution with 2% glucose and 2 ml of 2 U/ml of human insulin.

**ESR 1:** 2 ml of Tyrode solution with 2% glucose and 2 ml of 150 mg/ml of ESR.

**ESR 2:** 2 ml of Tyrode solution with 2% glucose and 2 ml of 300 mg/ml of ESR.

The test tubes were incubated at 37°C on a shaker at 140 cycles/minute for 30 minutes. Then, the amount of glucose in the original Tyrode solution (initial glucose concentration) and from the experiments (final glucose concentration) was determined using the glucose oxidase kit (Agappe Diagnostics). The amount of glucose absorbed per tissue was calculated as the difference between the initial and final concentration of glucose in the medium. The experiment was performed in triplicates.

In vivo effects of ESR on carbohydrate digestion in normal albino rats

Oral starch tolerance test

In this experiment, 15 normal non-diabetic Sprague Dawley rats of mass 200–250 g fasted overnight. Their fasting blood glucose (BG) was determined on a hand-held glucometer (Accu-check Active) after a tail puncture. Animals were randomly divided into three groups:

**NC:** 1 ml of distilled water

**ESR 1:** 150 mg/kg bw ESR

**ESR 2:** 300 mg/kg bw ESR

Animals were orally administered a starch solution (3 g/kg bw) [18] followed by the above treatments. BG level was then determined at periods 30, 60, 120, and 180 minutes to determine the effect of the extract on postprandial glycemia. The results were plotted on a graph and area under the curve (AUC) for each graph was determined based on the following equation.

\[
\text{AUC (mmol L⁻¹·h)} = \frac{BG_0 + BG_{30} \times 0.5}{2} + \frac{BG_{30} + BG_{60} \times 0.5}{2} + \frac{BG_{60} + BG_{120} \times 1}{2} + \frac{BG_{120} + BG_{180} \times 1}{2}
\]
Where BG represents blood glucose levels at time intervals 30, 60, 120, and 180 minutes [19].

**Oral sucrose tolerance test**

For this test, the same method used for starch tolerance test was used. 4 g/kg bw of sucrose was orally administered to rats in the place of starch.

**Statistical analysis**

All results were represented as mean \((n = 3)\) and SEM. Significance of experimental results was computed using two-way analysis of variance and results were considered significantly different at \(p < 0.05\).

**Results**

**Qualitative phytochemical analysis**

Percent yield of ESR obtained after 70% ethanol soaking was 8.24%. Phytochemical analysis showed positive results for many bioactive phytochemicals as shown in Table 1.

**ESR and α-Amylase inhibition**

ESR inhibited the activity of α-amylase with an increase in the concentration of ESR (Figure 1a). The highest percentage of inhibition of 56.7% was recorded at 1,000 µg/ml. The IC\(_{50}\) was 831.76 µg/ml. The mode of inhibition of the enzyme using a glucose standard curve (Figure 1b) and Lineweaver–Burk plot (Figure 1c) was determined as non-competitive inhibition. \(K_m\) and \(V_{max}\) were determined from the Lineweaver–Burk plot and are presented in Table 2.

**ESR and α-glucosidase inhibition**

ESR displayed α-glucosidase inhibition which increased steadily with an increase in the concentration of ESR, Figure 2a. The IC\(_{50}\), determined graphically was found to be 1202.3 µg/ml. From the Lineweaver–Burk plot (Figure 2b) and the kinetics constants (Table 2), the inhibition was concluded to be mixed non-competitive inhibition.

**ESR and glucose uptake by rat hemidiaphragms**

The effect of ESR on glucose uptake by rat hemidiaphragms is shown in Figure 3. Insulin and ESR significantly increased the uptake of glucose by the diaphragms. The ESR showed a significant dose-dependent effect on the uptake of glucose by the isolated rat hemidiaphragms.

**Effects of ESR on starch and sucrose tolerance tests**

The effect of ESR on the digestion of starch and sucrose in vivo was studied in this study using their tolerance tests in normal rats. The results are presented by graphs in Table 3, Figures 4 and 5. Compared to the NC administered distilled water, ESR exerted inhibitory effects on the digestion of both starch and sucrose to release glucose. ESR 1 (150 mg/kg bw) showed minimal inhibition with no significant \((P = 0.05)\) difference in comparison to the normal. However, ESR 2 (300 mg/kg bw) exerted a strong inhibition of both starch and sucrose digestion. ESR significantly \((P = 0.05)\) reduced peak BG levels and the AUCs for both starch and sucrose.

**Discussion**

The discovery and development of effective anti-diabetic drugs with less or no adverse side effects remains a challenge globally, hence, there is a much interest in botanicals. Botanicals seem to offer a better management of diabetes due to their holistic approach to the pathophysiology of the disease and few or no side effects. In the present study, the anti-diabetic activity of the ESR was evaluated together with its phytochemistry. Qualitative phytochemical screening showed that ESR contains phytochemical constituents like phenols, flavonoids, glycosides, tannins, saponins, and steroids. The results of the study are in agreement with the findings of Shaheen et al. [19] even though they used the methanol extract. These phytochemicals have been reported to have anti-oxidative and anti-diabetic [20] effects; hence, the traditional use of *Sida rhombifolia* in the management of diabetes complications. Phenols and flavonoids,

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**Table 1.** Phytochemical composition of ESR.

<table>
<thead>
<tr>
<th>Tested compound</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenols</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = present, − = absent

**Table 2.** The kinetic constants of α-amylase and α-glucosidase inhibition.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Control/ESR</th>
<th>(K_m) (mg/ml)(^{-1})</th>
<th>(V_{max}) (mg ml(^{-1}) S(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-amylase</td>
<td>Control</td>
<td>0.222</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>ESR</td>
<td>0.222</td>
<td>58.8</td>
</tr>
<tr>
<td>α-glucosidase</td>
<td>Control</td>
<td>0.17</td>
<td>71.4</td>
</tr>
<tr>
<td></td>
<td>ESR</td>
<td>0.2</td>
<td>52.6</td>
</tr>
</tbody>
</table>

ESR = extract of *Sida rhombifolia*
Figure 1. α-Amylase inhibition and mode of inhibition: (a) The percentage inhibition of α-amylase, (b) the glucose standard curve, and (c) the Lineweaver–Burk plot.
Figure 2. α-Glucosidase inhibition and mode of inhibition: (a) dose–response curve and (b) Lineweaver–Burk plot.

Table 3. Effect of ESR on AUC after starch and sucrose loading on normal rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Starch AUC (mmol/l.h)</th>
<th>% reduction of AUC</th>
<th>Sucrose AUC (mmol/l.h)</th>
<th>% reduction of AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>18.63 ± 0.06</td>
<td>–</td>
<td>21.15 ± 0.02</td>
<td>–</td>
</tr>
<tr>
<td>ESR 1</td>
<td>17.89 ± 0.32</td>
<td>3.97</td>
<td>20.21 ± 0.22</td>
<td>4.44</td>
</tr>
<tr>
<td>ESR 2</td>
<td>15.95 ± 0.25*</td>
<td>14.4</td>
<td>16.94 ± 0.02*</td>
<td>19.9</td>
</tr>
</tbody>
</table>

*P = 0.05 in comparison to NC
NC = normal control, ESR 1 = 150 mg/kg bw extract, ESR 2 = 300 mg/kg bw extract
The antidiabetic properties of Sida rhombifolia which are secondary metabolites, possess pharmacological properties like free radical scavenging activity, strong antioxidant activity, anti-inflammatory action, inhibition of hydrolyte, and oxidative enzymes [21]. Flavonoids inhibit α-glucosidase and aldose reductase thereby reducing postprandial BG level [21]. Tannins inhibit digestive enzymes like lipases, proteases, and glucosidases [18], the same mechanism of action used by other clinical synthetic drugs such as xenical and acarbose. Alkaloids and some saponins have BG reduction effect and antioxidant properties [22]. The phenolic and flavonoid richness of the extract may be responsible for the hypoglycemic activity of ESR.

The anti-diabetic effects of ESR were investigated by in vitro studies of α-amylase, α-glucosidase, and

Figure 3. Effects of ESR on glucose uptake by rat hemidiaphragms. *P = 0.05 in comparison to NC. NC = normal control, PC = positive control (insulin), ESR 1 = 150 mg/kg bw extract, ESR 2 = 300 mg/kg bw extract.

Figure 4. Effect of ESR on starch tolerance test in normal rats. NC = — Normal control, ESR 1 = —150 mg/kg bw extract, ESR 2 = —300 mg/kg bw extract.
rat hemidiaphragms. α-amylase is produced and released by the salivary glands and the pancreas to break starch into maltose and sucrose [22]. On the other hand, α-glucosidase found on the luminal surface of the small intestines breaks down disaccharides into the monosaccharide glucose for absorption into the bloodstream [23]. ESR significantly inhibited the catalytic activity of both α-amylase and α-glucosidase with IC₅₀ values of 831.76 and 1202.3 µg/ml, respectively. The inhibition of these enzymes by ESR was concentration dependent. This, therefore, shows that ESR plays a significant role in the management of postprandial hyperglycemia by slowing down the digestion of carbohydrates and their absorption into the bloodstream.

The inhibition of the mammalian α-glucosidase by Sida rhombifolia was also reported by Arciniegas et al. [24] with the acetone extract having the highest percentage inhibition than methanol and hexane. The inhibition of α-amylase and α-glucosidase by ESR may be linked to the presence of phenolic compounds such as flavonoids and tannins present in it [25].

The mode of inhibition of α-amylase and α-glucosidase by ESR was also investigated and determined on Lineweaver–Burk plots. It was concluded that ESR inhibited α-amylase in a non-competitive manner and α-glucosidase in a mixed non-competitive fashion as supported by $K_m$ and $V_{max}$. This implies that compounds in ESR do not bind to the substrate active sites of the enzymes; hence, inhibition cannot be overcome by increasing substrate concentration [26] an advantage over competitive inhibitors of same enzymes such as acarbose. The non-competitive inhibition of the carbohydrate hydrolyzing enzymes by ESR is like that of other reported plants extracts [27].

In vivo studies were conducted as confirmatory to in vitro results. It was found out that ESR at a dose of 300 mg/kg bw strongly reduced the peak BG concentrations and AUC under both starch and sucrose tolerance tests. This implies that after a carbohydrate meal where BG levels normally rise, ESR reduces them and maintains a steady glucose homeostasis. Henceforth, ESR is potentially suitable for the management of postprandial glycemia in type 2 diabetic patients. The reduction of postprandial glycemia implies that ESR inhibits α-amylase and α-glucosidase from digesting starch to maltose and sucrose, and sucrose to glucose, respectively [23]. This reduction confirms the inhibitory effects of ESR shown by the in vitro studies. Therefore, ESR can be attributed to have a similar mechanism of action to acarbose and miglitol, clinically used drugs for the management of postprandial diabetes [27].

Additionally, the ability of ESR to cause non-competitive inhibition of α-amylase and α-glucosidase

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**Figure 5.** Effect of ESR on sucrose tolerance test. NC = —normal control, ESR 1 = —150 mg/kg bw extract, ESR 2 = —300 mg/kg bw extract.
potentially makes it a better treatment option than the current standard of care for DM.

The effect of ESR on glucose uptake by muscle tissues was investigated using rat hemidiaphragms. ESR significantly promoted the uptake of glucose in a dose-dependent manner. ESR contains phytochemicals which may stimulate the expression of glucose transporters 4 (GLUT4) in muscle tissues [28], which aid in the efficient absorption of glucose. ESR may possess insulin-like properties which enhance uptake of glucose by resiping cells. ESR may also increase the endogenous production of insulin from pancreatic beta cells resulting in enhanced glucose uptake [29]. Because insulin sensitivity is impaired in type 2 DM, ESR may alleviate the sensitivity of the adipose and muscle tissues to the action of the circulating insulin. The increased peripheral uptake of glucose may contribute greatly to controlling postprandial hyperglycemia in type 2 DM.

Conclusion

It was concluded that ESR contains bioactive phytochemical constituents which may be responsible for its hypoglycemic activity. The anti-diabetic effects of ESR may be due to the inhibition of α-amylase and α-glucosidase, and increased uptake of peripheral glucose by muscle tissues. Therefore, ESR needs further research for potential use as an anti-diabetic drug.

Acknowledgments

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Conflict of Interest

We declare no conflict of interest.

References


