Phytochemical analysis and Hypoglycemic potential of *Scindapsus Officinalis* (Roxb.) Schott in Laboratory Animals via disaccharide absorption inhibition in GI

Nafisa Ferdous, Shehla Unaiza Hridi, J.M.A, Hannan and Md. Fakhruddin Mazumder

Department of Pharmacy, North South University, Plot -15, Block- B, Bashundhara R/A, Dhaka, Bangladesh.

**KEYWORDS**

Anti-diabetic; Anti-hyperglycemic; EESO; Hypoglycemic effect; Phytochemical; *Scindapsus officinalis*.

**ABSTRACT**

In this study, the ethanol extract of *Scindapsus officinalis* was first evaluated for phytochemical study. Since the compounds found in the fruit are of pharmacological interest, prompted us for its possible anti-diabetic activity. This research was primarily focused on the qualitative and quantitative evaluation of hypoglycemic effect of ethanolic extract of *Scindapsus officinalis* (EESO) fruit in laboratory animals and whether these effects were of any statistical significance. Qualitative phytochemical tests for the identification of alkaloids, flavonoids, steroids, gum and carbohydrates, reducing sugar, saponins, tannin and terpenoids were carried out for the plant extract with proper reagents. The fruit was further subjected to anti-diabetic study through six segment method and was investigated for anti-hyperglycemic effects in Long Evans rats. The anti-diabetic effects were estimated by measuring the amount of glucose in the samples, collected from six different segments of the gut, after the experiment. The amount of sucrose unabsorbed in different GIT segments were evaluated in control rats vs. rats fed with 500mg/kg extract at 30 minutes, 1 hour, 2 hour and 4 hour. Phytochemical analysis of ethanolic extract of *Scindapsus officinalis* has indicated the presence of steroid, carbohydrate, flavonoid, alkaloid, tanin, saponin and terpenoid-compounds. The extract caused a significant (p<0.05), dose dependent inhibition of glucose absorption and showed hypoglycemic effects in rats weighing from 80 – 200 gram. In conclusion, these observations provide evidence and possible mechanisms of action for the medicinal properties of fruit of *Scindapsus officinalis* claimed in Ayurveda medicine.

**Introduction**

Type 1 diabetes, defined by an absolute requirement for administration of exogenous insulin, results from the autoimmune destruction of the insulin
secreted pancreatic β cells. Type 1 diabetes is a severe form associated with ketosis in the untreated state. It arises most commonly in juveniles but occasionally in non-obese adults and elderly. It is a catabolic disorder in which circulating insulin is virtually absent with elevated level of plasma glucagon. Exogenous insulin is therefore required to reverse the catabolic state, prevent ketosis and reduce the elevated blood glucose level. Type 2 or non-insulin-dependent diabetes mellitus is characterized (American Diabetes Association, 2001) by a relative insulin deficiency due to predominantly an insulin secretory defect with insulin resistance.

Type 2 diabetes represents a heterogeneous group of disorders comprising milder forms of diabetes that occur predominantly in adults but occasionally in 21 adolescents. Circulating exogenous insulin is sufficient to prevent ketoacidosis but is often either subnormal or relatively inadequate because of tissue insensitivity (Rodger 1991).

In traditional practice medicinal plants are used in many countries to control diabetes mellitus. The hypoglycaemic action of these medicinal plants are being studied (Alarcon-Aguilara et al., 1993). Plant drugs are frequently considered to be less toxic and more free from side effects than synthetic ones (Pari and Umamaheswari, 2000). In the traditional system of Indian medicine plant formulation and in several cases, combined extracts of plants are used as the drug of choice rather than individual. Many of these have shown promising effects (Kumari and Devi, 1993).

*Scindapsus officinalis* (Roxb.) Schott (Aracea) is a monocotyledonous medicinal climber (growing along the sub-Himalayan tract in West Bengal, Andhra Pradesh and the Andaman Islands) which has endowed with curative properties against a variety of illness. The folk lore claim of *Scindapsus officinalis* fruits are anti-diabetic, anthelmintic, aphrodisiac, galactagogue, stimulant, diaphoretic, antidiarrhoeal, carminative, expectorant, tonic, antiprotozoal, anticancer, sharpening hearing, aphrodisiac, cardio tonic and regulating the bowel and appetite. It is also used in dysentery, asthma, troubles of the throat, rheumatism, asthma, worm infestations, pharyngopathy, helminthiasis and bronchitis (Patel et al., 2010). Various pharmacological activities on fruit part of plant (like antioxidant, anti-inflammatory, analgesic, antihistaminic, antibacterial) have been scientifically reported. Hence, the present studies were undertaken to find out the phytochemical components and possible anti-diabetic activity of ethanolic extract of *Scindapsus officinalis* fruit using- six segment method in long evans rat respectively.

**Materials and Methods**

Hot Plate (Model – 35100, UGO BASILE, ITALY), Electronic Balance, Refrigerator, Beakers, Petri dishes & glass wrought, Safety rat handling gloves, Mortar & pestle, Hypodermic Syringes, Holder & test tube, Glucose kit, Vortex, Centrifuge, Homogenizer, Screw-cap test tubes, Surgical apparatus (forceps, scissors), Micropipette, Incubator, UV-Spectrophotometer, Sonicator.

**Medicinal plant (extract)**

Extract was examined in one concentration of 500mg/kg body weight of animal

**Reagent, Control and Positive Control Phytochemical Study**

Reagents and chemicals, Wagner Reagent, concentrated HCL, 0.1% Ferric chloride,
Molish reagent, conc. H$_2$SO$_4$, α-naphthol, chloroform

**Anti-diabetic activity Six Segment**

Control- Sucrose solution, Reagents- NaOH(1N), H$_2$SO$_4$(2N), Ketamine, Ice cold saline, 80% ethanol

**Plant Extraction method**

**Collection**

The plant sample of *Scindapsus officinalis* was collected from an Ayurvedic Institution ‘Back to Nature’ on 18.06.2012 in the form of fruit shavings. The fruit of the plant was procured and cleansed with water several times to rinse away dirt and undesirable materials.

**Drying and grinding**

The collected fruit was washed with water, separated from undesirable materials or plant parts, partially dried by fan aeration and then fully dried in the oven at below 40°C for 2 days. The fully dried fruit was then grinded and coarsely powdered. The powdered form was stored in the refrigerator at +4°C for a few days.

**Cold extraction (Ethanol extraction)**

542gm of powdered plant material was then dissolved in 80% ethanol. The alcoholic mixture was sealed in a conical flask and placed on a shaker for 2 days period to receive occasional shaking and stirring. Subsequently, the homogenized mixture was subjected to a coarse filtration by a piece of clean, white cotton material. The primary filtrate underwent a second phase of filtration through whatman filter paper. The filtrate (Ethanol extract) obtained was evaporated by Rotary evaporator (Eyela n 1000, Tokyo Rikaki kai co.ltd, Rotary vacuum, Japan) at 4 to 5 rpm and at 65°C temperature. It rendered a gummy concentrate of dark brown color, designated as the crude extract of *S.Officinalis*. Then the crude extract was dried by freeze drier and preserved at 4°C. The preserved extract was later subjected to biological screening and pharmacological experiments.

**Phytochemical Analysis**

**Study Design**

Qualitative phytochemical tests for the identification of alkaloids, flavonoids, steroids, gum and carbohydrates, reducing sugar, saponins, tannin and terpenoids were carried out for the plant extract by the method described by Harborne and Sazada (Harborne, 1998 and Sazada et al, 2009).

The freshly prepared extract of *Scindapsus Officinalis* was qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extract was performed using the following reagents and chemicals: Alkaloids with Wagner reagent, flavonoids with the use of conc HCl, tannins with 0.1% ferric chloride, and saponins with ability to produce suds. Gum was tested using Molish reagents and concentrated sulfuric acid, steroids with sulfuric acid, reducing sugar with the use α-napthol and sulfuric acid and terpenoids with chloroform and conc. HCl (Aguinaldo et al, 2005).

**Antidiabetic activity**

**Experimental Animal**

Both genders of Long Evans rats (*Rattus norvigicus*) were selected for the present study and acclimatized under standard conditions. Long Evans rats (male and
female), weighing 80-200g of either sex were collected from ICDDRBR for the study and were kept in standard environmental condition for weeks in the animal house of the Department of Pharmacy, North south University, Bangladesh for adaptation after their purchase. The animals were housed under standard laboratory conditions (relative humidity 55-65%, room temperature of 25.0 ± 2°C, and 12 hrs light dark cycle). The animals were fed with standard diet (pellets) and had free access to filtered water (M.K Sharif et al, 2011).

**Six Segment methods for the assessment of Anti-diabetic activity**

Plant extract (500mg/kg) along with sucrose solution (2.5g/kg body weight) were administered orally to 24 hours fasted rats. Control group was given equal volume of sucrose only. Ketamine hydrochloride was injected intraperitonially 15 minutes prior to dissection of rats of each hour (30min, 1hr, 2hr & 4hr) to elicit acute anesthetic effect and eventually death. For 30, 60, 180 and 360 minutes respective rats were sacrificed. After sacrificing, the whole GIT was excised into six segments. The segments being – (A) Stomach, (B) Upper 20 cm of small intestine, (C) Middle part of small intestince, (D) Lower 20 cm of small intestine, (E) Caecum and (F) Large intestine. Each segment was then washed with 10 ml of ice cold saline. The solution was then centrifuged for 15 minutes at 3000 rpm. The supernatant was then collected and to this solution, 2N H_2SO_4 (2ml) was added to acidify the solution. These mixtures were then boiled for 2 hours in paraffin oil to hydrolyse the sucrose. After 2 hours, to these mixtures, 1N NaOH was added drop by drop to neutralize the mixture and the pH was set at 6.9-7.

Then the concentration of glucose was obtained by the use of GOD-PAP method and ELISA reader. Blood glucose and the amount of glucose liberated from residual sucrose in the gastrointestinal tract were measured. The gastrointestinal sucrose content was calculated from the amount of liberated glucose (Goto et al, 1995).

**Calculation of sucrose from glucose**

Amount of sucrose in certain volume = C x V x 0.342

Here, C= Conc. Of glucose (mmol/ l)
V= Total volume of solution

**Results and Discussion**

**Phytochemical screening**

Phytochemical screening of the ethanolic extract of *Scindapsus officinalis* fruit revealed the presence of various bioactive components such as tannins, flavonoids, saponins, gums, steroids, alkaloids, reducing sugar and terpenoids. The result of phytochemical test has been summarized in the table 1.

**Anti-diabetic Activity (Six-segment method)**

In six segment method, sucrose and extract solution were administered to the model rats, and water and sucrose were administered to the control. Then after 30 minutes, 60 minutes, 120 minutes and 240 minutes the rats were sacrificed to observe the amount of sucrose remaining in the gastrointestinal tract. Results are expressed as (mean value± SD) in mg. Administration of ethanolic extract of *Scindapsus officinalis* (0·5 g/kg) with the sucrose load in rats increased the residual intestinal sucrose content significantly (P<0·05). The
total sucrose content remaining in the gastrointestinal tract was increased in *S.officinalis* extract treated rats compared with normal controls.

From the result we can deduce that the extract of the fruit of *S.officinalis* was able to cause a decrease in the absorption of sucrose solution from the gastrointestinal tract and elicit hypoglycemia.

**Acute toxicity**

Oral administration of graded doses (500mg/kg) of the ethanol extract of *Scindapsus officinalis* to rats did not produce any significant changes in behaviour, breathing, cutaneous effects, sensory nervous or systemic responses during the observation period. No mortality was recorded in any group after 24h of administering the extract to the animals. Results are expressed as mean ±SEM, N=6 for all experiments. Analyses were performed using one-way ANOVA by SPSS Software 17.00 version. P values of 0.05 or less were considered as significant.

Isolation of pure, pharmacologically active constituents from plants remains a long and tedious process. For this reason, it is necessary to have methods available which eliminate unnecessary separation procedures. Chemical screening is thus performed to allow localization and targeted isolation of new or useful constituents with potential activities. This procedure enables recognition of known metabolites in extracts or at the earliest stages of separation and is thus economically very important (Kumara , 2001 and Lippincott’s, 3rd edition).

Preliminary qualitative phytochemical screening of *S.officinalis* fruit extract exhibited the presence of alkaloids, carbohydrates and gums, flavonoids, reducing sugars, saponin and terpenoids. Therefore it is assumed that these compounds may be responsible for the observed analgesic activity. Flavonoids were reported to have a role in analgesic activity primarily by targeting prostaglandins (Rajnarayana *et al.*, 2001, Rao *et al.*, 1998).

There are also reports on the role of tannins in anti-nociceptive activity (Vanu *et al.*, 2006). Besides, alkaloids are well known for their ability to inhibit pain perception (Uche and Aprioku, 2008). Flavonoids and other phenolic compounds of plant origin have been reported as antioxidants and as scavengers of free radicals. Antioxidants can also exert anti-inflammatory effects (Ferrandiz and Alcaraz, 1991). The flavonoids and tannins have been reported to produce anti-diabetic activity (Suba *et al.*, 2004).

The present study was undertaken to investigate the hypo-/antihyperglycemic activity of *S.officinalis* extract in non-diabetic rats. Hypoglycemic activity that is found when given with a simultaneous glucose load in diabetic rats indicates that the extracts may interfere with the intestinal glucose absorption in the GI by various mechanisms (Nahar *et al.*, 2000). One of the objectives of the study was to investigate whether the hypoglycemic effect is related to the inhibition of glucose absorption in the GI. From the result we can deduce that the extract of the fruit of *S. officinalis* was capable of causing a decrease in the absorption of sucrose solution from the gastrointestinal tract. This anti-diabetic property can be linked with the ability of the polyphenolic tannins and flavonoids (present in the fruit extract) to inhibit α-glucosidase enzyme (Chakravarthy *et al.*, 1982, Caspary and
Table 1: Result of Phytochemical Screening of Plant Extract

<table>
<thead>
<tr>
<th>S. officinalis Extract</th>
<th>Tannins</th>
<th>Saponins</th>
<th>Flavonoids</th>
<th>Gums &amp; Carbohydrates</th>
<th>Alkaloids</th>
<th>Reducing Sugars</th>
<th>Terpenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>80% ethanol</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

Table 2: Anti-diabetic activity of Scindapsus officinalis (500mg) in Upper Intestine

<table>
<thead>
<tr>
<th>Group</th>
<th>30mins</th>
<th>1hr</th>
<th>2hrs</th>
<th>4hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23.19±1.68</td>
<td>17.21±1.17</td>
<td>12.71±1.06</td>
<td>6.28±1.35</td>
</tr>
<tr>
<td>Scindapsus500mg</td>
<td>50.68±1.89</td>
<td>37.31±2.31</td>
<td>24.75±1.59</td>
<td>14.86±1.64</td>
</tr>
</tbody>
</table>

Figure 1: Anti-diabetic activity of S. officinalis in Upper Intestine

Table 3: Anti-diabetic activity of Scindapsus officinalis (500mg) in Middle Intestine

<table>
<thead>
<tr>
<th>Group</th>
<th>30mins</th>
<th>1hr</th>
<th>2hrs</th>
<th>4hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18.98±.97</td>
<td>13.00±.52</td>
<td>10.02±.70</td>
<td>4.66±.29</td>
</tr>
<tr>
<td>Scindapsus500mg</td>
<td>30.81±1.18</td>
<td>18.94±1.08</td>
<td>15.77±1.21</td>
<td>12.12±.71</td>
</tr>
</tbody>
</table>
Figure 2: Anti-diabetic activity of *S. officinalis* in Middle Intestine

**Table 4** Anti-diabetic activity of *Scindapsus Officinalis* (500mg) in Lower Intestine

<table>
<thead>
<tr>
<th>Group</th>
<th>30mins</th>
<th>1hr</th>
<th>2hrs</th>
<th>4hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.75±.96</td>
<td>16.33±1.18</td>
<td>10.34±.56</td>
<td>5.91±.51</td>
</tr>
<tr>
<td>Scindapsus500mg</td>
<td>9.61±.33</td>
<td>19.37±.68</td>
<td>14.64±1.03</td>
<td>10.08±.86</td>
</tr>
</tbody>
</table>

**Table 5** Anti-diabetic activity of *Scindapsus officinalis* (500mg) in Stomach

<table>
<thead>
<tr>
<th>Group</th>
<th>30mins</th>
<th>1hr</th>
<th>2hrs</th>
<th>4hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>31.08±1.93</td>
<td>25.53±1.52</td>
<td>15.75±.61</td>
<td>6.32±1.73</td>
</tr>
<tr>
<td>Scindapsus500mg</td>
<td>62.46±2.72</td>
<td>47.56±1.30</td>
<td>36.03±1.46</td>
<td>18.54±2.25</td>
</tr>
</tbody>
</table>

**Table 6** Anti-diabetic activity of *Scindapsus officinalis* (500mg) in Cecum

<table>
<thead>
<tr>
<th>Group</th>
<th>30mins</th>
<th>1hr</th>
<th>2hrs</th>
<th>4hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.90±.43</td>
<td>20.47±.91</td>
<td>13.91±.99</td>
<td>7.08±.48</td>
</tr>
<tr>
<td>Scindapsus500mg</td>
<td>8.77±.63</td>
<td>22.60±1.00</td>
<td>22.29±1.14</td>
<td>9.25±.41</td>
</tr>
</tbody>
</table>

**Table 7** Anti-diabetic activity of *Scindapsus officinalis* (500mg) in Large Intestine

<table>
<thead>
<tr>
<th>Group</th>
<th>30mins</th>
<th>1hr</th>
<th>2hrs</th>
<th>4hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.74±.963</td>
<td>16.33±1.18</td>
<td>10.33±.560</td>
<td>5.91±.511</td>
</tr>
<tr>
<td>Scindapsus500mg</td>
<td>9.60±.334</td>
<td>19.37±.679</td>
<td>14.63±1.03</td>
<td>10.33±.653</td>
</tr>
</tbody>
</table>

**Table 8** Anti-diabetic activity of *Scindapsus officinalis* (500mg) in Total GIT

<table>
<thead>
<tr>
<th>Group</th>
<th>30mins</th>
<th>1hr</th>
<th>2hrs</th>
<th>4hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>92.33±2.58</td>
<td>99.81±5.24</td>
<td>75.05±1.81</td>
<td>41.07±6.43</td>
</tr>
<tr>
<td>Scindapsus500mg</td>
<td>175.62±9.82**</td>
<td>142.52±10.74**</td>
<td>131.38±3.26***</td>
<td>74.79±2.01***</td>
</tr>
</tbody>
</table>
Creutzfeld, 1971). The ethanolic extract showed significant dose dependent inhibition in activity compared with the controls.

Since glucose lowering effect of \textit{S. officinalis} was clearly evident from previous study reports and claims, glucose absorption inhibition could have been a possible mechanism responsible for the hypoglycemic effect (Lembeke, 1987; Swintosky and Pogonowskawala, 1982). Our study confirms this effect, because when ethanolic extract of \textit{S. officinalis} was given along with sucrose solution it significantly increased sucrose retention in the gut compared with only sucrose solution fed control group of rats.

In conclusion, the present study demonstrated that the ethanolic extracts of \textit{Scindapsus Officinalis} showed significant inhibition of carbohydrate digestion and absorption, which has resulted in the well-known hypoglycemic effects of the fruit extract (Cotton, 1996; Balick and Cox, 1996).

Acknowledgement

We would like to express our utmost sincere gratitude and respect to Associate Professor Dr. JMA Hannan, former Chairman, Department of Pharmacy, North South University, our honorable teacher and thesis supervisor, for his active encouragement and relentless support. He has been a constant support and inspiration for us to accomplish this work. We would also like to convey our gratitude and appreciation to Md. Fakhruddin Mazumder, Lab officer, for guiding and helping us during our research work. Finally we would like to acknowledge all the honorable officilas and staffs of the Department of Pharmacy, North South University, Bashundhara, Dhaka, Bangladesh.

References


Kumara, N.K.V.M.R., 2001. Identification of strategies to improve research on
Lippincott’s Illustrated Reviews : Pharmacology : 3rd edition