Research Journal of Pharmaceutical, Biological and Chemical Sciences

Standardization and Quality Evaluation of a Traditional Antidiabetic Polyherbal Formulation Sugnil.

Paranthaman Karthikeyan¹*, Gopal Sathiya¹ and Subbramanium Sridhar²

¹Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalainagar–608 002, Tamil Nadu, India.
²Ashram Siddha Yoga Research Institute, Salem-636004, Tamil Nadu, India.

ABSTRACT

Herbal drug technology is used for converting botanical materials into medicines, where standardization and quality control with proper integration of modern scientific techniques is important. In the present study, physio-chemical analysis, screening for microorganisms, primary and secondary metabolites analysis, heavy metal analysis, HPTLC analysis of bioactive marker compounds and invitro α-glucosidase inhibitory effect were carried out as a part of standardization and quality evaluation of sugnil. Physiochemical analysis gives surety about the product which is genuinely prepared. Microbial screening shows that there was no microbial contamination in the product and thus, assures its quality and presumed safety. Nutritional assessment indicates its good nutritional value. Secondary metabolite analysis reveals the presence of active phytochemicals which are mainly involved in the pharmacological actions of sugnil. Heavy metal analysis confirms its non-toxicity during the course of treatment. HPTLC finger print indicates that the product sugnil was prepared from genuine plants or parts of the plants and presence of major bioactive compounds assures the genuiness of the formulation. The strong α-glucosidase inhibitory activity of sugnil substantiates its potent invitro antidiabetic activity. Overall, the results show that the traditional Siddha antidiabetic polyherbal formulation sugnil is genuine and a standardized drug.

Keywords: Sugnil, HPTLC, Physiochemicals, α-glucosidase.

*Corresponding author
INTRODUCTION

Popularity and increasing demand of herbal medicines create an en route for founding of many herbal drug-manufacturing units in developing countries like India. It is estimated that there are over 7800 medicinal drug-manufacturing units in India. The major problem faced by the herbal drug industry is non-availability of rigid quality control profile for herbal material and their formulations. The quality control of crude drugs and herbal formulations is of paramount importance in justifying their acceptability in modern system of medicine. Hence, it is necessary to develop simple bioassays for biological standardization to ensure the quality of the herbal drugs [1]. Besides, qualitative and quantitative analysis of major bioactive chemical components (marker components) of herbal drug constitutes an important and reliable part of quality control protocol.

Sugnil, a traditional antidiabetic polyherbal formulation consists of ingredients from nine medicinal plants viz Aristolochia bracteata, Balsamodendron mukul, Casearia esculanta, Cassia auriculata, Coscinium fenestratum, Curcuma longa, Eugenia jambolana, Gymnema sylvestre, and Triphala. Preparation of Sugnil is based on traditional methods in accordance with the procedure suggested in the antique literature. This drug is being widely used by many Siddha medicinal practitioners for more than 15 years to treat diabetic patients and found to be effective in the management of diabetes and its related complications. However, still there is no scientific report on quality evaluation and standardization of this formulation. This drug was therefore selected for the present study in order to assess its quality through invitro assays.

Sugnil was received as a gift sample from herbal drug manufacturing unit ‘Naturo Herbal Remedies’, Salem, Tamilnadu. The guidelines of World health organization (WHO) and Central council of research in Ayurveda and Siddha (CCRAS) were followed in the standardization of sugnil formulation. Physio-chemical analysis, screening for microorganisms, primary and secondary metabolites analysis, heavy metal analysis and HPTLC analysis of bioactive marker compounds were carried out as a part of standardization and quality evaluation of sugnil. The assessment of invitro α-glucosidase inhibitory effect of sugnil was also included in the study.

METHODOLOGY

Physio-Chemical Analysis

Total Ash

A weighed amount of the powder was taken in a silica crucible previously ignited, cooled and weighed. It was incinerated using incinerator by gradually increasing the heat not exceeding dull red heat (450°C) until free from carbon, cooled and weighed. The percentage of ash was calculated with reference to air-dried drug. The procedure was repeated to get constant weight.
Water Soluble Ash

The total ash was boiled with 25 ml water and filtered through ash less filter paper (Whatmann 4.1). It was followed by washing with hot water. The filter paper was dried and ignited in a silica crucible, cooled and the water insoluble ash was weighed. The water-soluble ash was calculated by subtracting the water insoluble ash from the total ash.

Acid Insoluble Ash

The total ash obtained was boiled for 5 minutes with 25 ml of dilute hydrochloric acid (10% w/v) and filtered through ash less filter paper (Whatmann No.1). The filter paper was ignited in a silica crucible, cooled and weighed.

Microbial Analysis

A stock solution of sample was prepared (1g in 25 ml of 0.1 % sterile peptone water). After serial dilution, the sample was poured onto specific media and incubated under appropriate conditions for microbial testing, according to Bergey’s manual. The media used were soybean casein digest broth medium (SCDM), soybean casein digest agar (SCDA), cetrimide agar medium (CAM).

Quantitative Assays

Preparation of extract

Accurately 1g of sugnil was extracted with 90 ml of methanol for 6 hrs in a Soxhlet apparatus. The extract was then concentrated at a temperature below 50°C, filtered through Whatman filter paper No1 and the final volume was made up to 100 ml with methanol in a volumetric flask. 10 ml of this solution was evaporated to dryness on a water bath and once again dissolved in 2 ml of methanol. This methanolic extract of sugnil was used as a test sample for primary and secondary metabolite analysis and high performance thin layer chromatography (HPTLC) analysis.

Analysis of Primary Metabolites

The protein and carbohydrate contents in sugnil were determined by Lowry et al and anthrone method respectively. The content of total steroids was also determined.

Analysis of Secondary Metabolites

A colorimetric method using aluminum chloride was employed for flavonoids determination (Chang et al., 2002). Total tannin content was determined by the method of Schanderl, 1970. Total phenol content was determined using Folin-Ciocalteau reagent by the method of McDonald et al., 2001.
Elemental Analysis by Atomic Absorption Spectrophotometry

About 250 mg of sugnil was weighed and 5-10 ml of concentrated sulphuric acid was added to it. The acid digestion was further initiated by heating upto 440°C using a digesdahl apparatus. The samples were made free of organic matter and the resulting solution was made colourless by adding 5-10 ml of H₂O₂. The digested material was made up to 100 ml for elemental analysis in the AAS, Perkin Elmer. Hg and Se were estimated using a hydride generator attached to the AAS. Working standard solutions were prepared from stock standard solutions. Calibration was performed using appropriate standard solutions. Results were arrived at from standard linear calibrations.

HPTLC Analysis of Marker Compounds

Equipment

A Camag HPTLC system equipped with a sample applicator Linomat V, twin trough plate development chamber, TLC Scanner III, Reprostar and Wincats 4.02, integration software (Switzerland) was used to identify the active components present in the methanolic extract of sugnil.

Chemicals

Analytical reagent grade toluene, ethyl acetate, methanol, acetic acid, n-hexane, chloroform and formic acid were obtained from SD Fine Chem Ltd. (Mumbai, India). Pure ellagic acid, gallic acid, curcumin and gymnemic acid were obtained from Natural Remedies Ltd., (Bangalore, India) as gift samples. Pre-coated silica gel 60 F₂₅₄ TLC aluminium plates (10x10 cm, 0.2 mm thick) were obtained from E. Merck Ltd. (Mumbai, India).

Preparation of Standard Solutions

Gallic Acid

A stock solution of gallic acid (1 mg mL⁻¹) was prepared by dissolving 10 mg of accurately weighed gallic acid in methanol and making up the volume to 10 ml with methanol. The stock solution was further diluted with methanol to give a standard solution of gallic acid (250 μg mL⁻¹).

Ellagic Acid

A stock solution of ellagic acid (100 μg mL⁻¹) was prepared by dissolving 10 mg of accurately weighed ellagic acid in methanol and making up the volume to 100 ml with methanol. The stock solution was further diluted with methanol to give a standard solution of ellagic acid (25 μg mL⁻¹).
Curcumin

A stock solution of curcumin (100 μg mL⁻¹) was prepared by dissolving 10 mg of accurately weighed curcumin in methanol and making up the volume to 100 mL with methanol. The stock solution was further diluted with methanol to give a standard solution of curcumin (50 μg mL⁻¹).

Gymnemic Acid

A stock solution of gymnemic acid (100 μg mL⁻¹) was prepared by dissolving 10 mg of accurately weighed gymnemic acid in methanol and making up the volume to 100 mL with methanol. The stock solution was further diluted with methanol to give a standard solution of gymnemic acid (50 μg mL⁻¹).

Chromatographic Conditions

**Sugnil Extract**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Polyherbal Formulation sugnil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample prepared in</td>
<td>Methanol</td>
</tr>
<tr>
<td>Stationary phase</td>
<td>Silica gel GF₂₅₄</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>nHex:EA:FA:GAA (15:6:0.5:1.5)</td>
</tr>
<tr>
<td>Scanning wavelength</td>
<td>300 nm</td>
</tr>
<tr>
<td>Sample concentration</td>
<td>10mg/ml</td>
</tr>
<tr>
<td>Applied volume</td>
<td>Track 1(5µl) and Track 2 (10µl)</td>
</tr>
<tr>
<td>Development mode</td>
<td>Ascending mode</td>
</tr>
</tbody>
</table>

**Gallic Acid**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Polyherbal Formulation sugnil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>Gallic Acid (GA)</td>
</tr>
<tr>
<td>Sample prepared in</td>
<td>Methanol</td>
</tr>
<tr>
<td>Stationary phase</td>
<td>Silica gel GF₂₅₄</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Tol:EA:FA:M (3:3:0.8:0.2)</td>
</tr>
<tr>
<td>Scanning wavelength</td>
<td>280nm</td>
</tr>
<tr>
<td>Sample concentration</td>
<td>Sugnil (I-10mg/ml), GA (II-250 μg/ml)</td>
</tr>
<tr>
<td>Applied volume</td>
<td>Track 1(5 µl) anf Track 2 (2.5 µl)</td>
</tr>
<tr>
<td>Development mode</td>
<td>Ascending mode</td>
</tr>
<tr>
<td>Lamp</td>
<td>Deuterium</td>
</tr>
</tbody>
</table>

**Ellagic Acid**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Polyherbal Formulation sugnil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>Ellagic Acid (EA)</td>
</tr>
</tbody>
</table>
Sample prepared in - Methanol
Stationary phase - Silica gel GF$_{254}$
Mobile phase - Tol:EA:FA:MeOH (3:3:0.8:0.2)
Scanning wavelength - 280nm
Sample concentration - Sugnil (I-10mg/ml), EA (II-25 µg/ml)
Applied volume - Track 1(10 µl) and Track 2 (10 µl)
Development mode - Ascending mode
Lamp - Deuterium

**Curcumin**

Sample - Polyherbal Formulation sugnil
Standard - Curcumin (CR)
Sample prepared in - Methanol
Stationary phase - Silica gel GF$_{254}$
Mobile phase - CHcl$_3$: Ethanol: AA (95:5:1)
Scanning wavelength - 366 nm
Sample concentration - Sugnil (I-10mg/ml), CR (II-50 µg/ml)
Applied volume - Track 1(10 µl) and Track 2 (5 µl)
Development mode - Ascending mode
Lamp - Deuterium

**Gymnemic Acid**

Sample - Polyherbal Formulation sugnil
Standard - Gymnemic acid (GYA)
Sample prepared in - Methanol
Stationary phase - Silica gel GF$_{254}$
Mobile phase - nHex: EA: AA (33:14:5)
Scanning wavelength - 254 nm
Sample concentration - Sugnil (I-20mg/ml), GYA (II-50 µg/ml)
Applied volume - Track 1(10 µl) and Track 2 (5 µl)
Development mode - Ascending mode
Lamp - Deuterium

**Instrumentation and Procedure**

Chromatography was performed on precoated silica gel GF$_{254}$ HPTLC plates (10x10 cm, 0.2 mm thickness). The plates were pre-washed with methanol and dried in an oven at 105°C for 2 h. Methanolic extract of sugnil and standard solutions of gallic acid, ellagic acid, curcumin and gymnemic acid were spotted separately on a 10x10 cm precoated TLC plates as 6 mm wide band and 8 mm from the bottom by using automatic TLC applicator Linomat V. The plates were developed in a twin trough chamber, under the respective chromatographic conditions given above, by ascending mode to a distance of 8 cm under chamber saturation conditions. After
development the plates were dried in air and scanned at 300 nm for sugnil extract, 280 nm for gallic acid and ellagic acid, 366 nm for curcumin and 254 nm for gymnemic acid using Camag Scanner III. The plates were photographed at 254 and 366 nm using Camag Reprostar instrument. The contents of gallic acid, ellagic acid, curcumin and gymnemic acid in the methanolic extract of V were calculated from the respective calibration curve. The results were generated using HPTLC software Win CATS 1.4.4.6337.

**Determination of α-glucosidase Inhibitory Activity**

The inhibitory activity was determined by incubating a solution of 1 ml of 2% starch substrate with 0.2 M Tris buffer pH 8.0 and various concentrations of sugnil for 5 min at 37°C. The reaction was initiated by adding 1 ml of α-glucosidase enzyme (1U/ml) followed by incubation at 37°C for 30 mins. Then, the mixture was heated in boiling water bath to stop the reaction. The amount of liberated glucose was measured by glucose oxidase peroxidase method. All determinations were carried out in triplicate. Meglitabose was used as a positive control.

**Calculation of 50% Inhibitory Concentration (IC_{50})**

The concentration of drug required to inhibit 50% of the enzyme (IC_{50}) was calculated by using the percentage scavenging activity at different concentrations of the drug. Percentage inhibition (I %) was calculated by I % = (A_C-A_S)/A_C X 100, where A_C is the absorbance of the control and A_S is the absorbance of the sample.

**RESULTS AND DISCUSSION**

**Physio-chemical analysis of sugnil**

Sugnil was tested for relevant physio-chemical parameters. The result revealed that total ash content (13.55% w/w), acid soluble ash (6.85% w/w) and water soluble ash (6.85% w/w) were within standard range (Table 1). A high ash value and less water acid-soluble extractive value are indicative of contamination, substitution, adulteration or carelessness in preparing, processing and storage of the drug for marketing. The data obtained from sugnil analysis with respect to physiochemical parameters were found to be well within the standard range and shows that there are very few impurities in the product. It also gives surety about the product which is genuinely prepared.

**Microbial analysis of sugnil**

Microbiological screening was done for sugnil (Table 2). Pathogens like E.coli, S. aureus, P. aeruginosa and salmonella were found to be absent. TAMC (620 Cfu/g) and TYMC (93 Cfu/g) were found to be within limits. The absence of pathogens in the product assures the quality of SUGNIL and also its presumed safety.
Table 1: Physicochemical and Phytochemical analysis of Sugnil
(Values are mean of three determinations ± SEM)

<table>
<thead>
<tr>
<th>Physiochemical analysis</th>
<th>Phytochemical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash content</td>
<td>Sugnil (%)w/w</td>
</tr>
<tr>
<td>Total ash</td>
<td>Carbohydrates (mg/g)</td>
</tr>
<tr>
<td>Acid soluble</td>
<td>Steroids (%)w/w</td>
</tr>
<tr>
<td>Water soluble</td>
<td>Proteins (mg/g)</td>
</tr>
</tbody>
</table>

Table 2: Microbial Analysis of Sugnil

<table>
<thead>
<tr>
<th>S.No</th>
<th>Microorganisms</th>
<th>Absent/ Present</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>E. coli</td>
<td>Absent</td>
</tr>
<tr>
<td>2</td>
<td>S. Aureus</td>
<td>Absent</td>
</tr>
<tr>
<td>3</td>
<td>P. Aeraginosa</td>
<td>Absent</td>
</tr>
<tr>
<td>4</td>
<td>Salmonella</td>
<td>Absent</td>
</tr>
<tr>
<td>5</td>
<td>TAMC</td>
<td>620 Cfu/gm</td>
</tr>
<tr>
<td>6</td>
<td>TYMC</td>
<td>93 Cfu/gm</td>
</tr>
</tbody>
</table>

TAMC – Total aerobic microbial count; TYMC – Total yeast microbial count; Cfu – Colony forming units.

Phytochemical analysis of sugnil

Sugnil contained good amount of carbohydrates (105mg/g), proteins (28mg/g) and steroids (78mg/g) (Table 1). Presence of these nutrients at high amounts proves the nutritional value of SUGNIL. Phytochemicals in sugnil were identified after analysis for secondary metabolites (Table 1). Active phytoconstituents like flavonoids (11.09%w/w), tannins (16.54%w/w) and phenols (17.74%w/w) were present.

Heavy metal analysis of Sugnil

Sugnil was analyzed for presence of heavy metals using AAS (Table 3). The heavy metals with their respective amounts are: Fe-0.855ppm; Zn-0.018ppm; Cu-1.977ppm; Mn-0.129ppm; Cr-0.015ppm; Pb-0.068ppm; As-not detected; Hg-1.344ppm; Se-not detected; and Co-0.042ppm. The values obtained are well within the acceptable limits suggesting that sugnil is nontoxic and suitable for prescription to humans.

Table 3: Elemental Analysis of Sugnil

<table>
<thead>
<tr>
<th>Sample</th>
<th>Elements</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fe (ppm)</td>
</tr>
<tr>
<td>Sugnil</td>
<td>0.885</td>
</tr>
</tbody>
</table>

*ND – Not detected
HPTLC Analysis of Sugnil

The qualitative and quantitative analysis of major bioactive chemical compounds (marker compounds) in crude drug constitutes important and reliable part of quality control. Therefore, the important marker compounds such as gallic acid (GA), ellagic acid (EA), curcumin (CR) and gymnemic acid (GYA) in sugnil were quantitatively estimated by HPTLC analysis.

HPTLC finger print of sugnil is depicted in Figure 1a. The peaks in the chromatogram indicate the presence of a number of active ingredients in the formulation in proposed quantity without any impurity. The well resolved HPTLC chromatogram of the methanol extract of SUGNIL, together with the reference standards such as GA, EA, CR and GYA are described in Figures 1b, 1c,1d and 1e respectively. The peaks with Rf values 0.55, 0.47, 0.58 and 0.55 in the chromatogram of the methanolic extract of sugnil correspond to GA, EA, CR and GYA respectively. Standards such as GA, EA, CR and GYA showed single peaks in respective HPTLC chromatograms with the maximum Rf values of 0.56, 0.51, 0.56 and 0.53 respectively (Fig. 2a - 2d). To ascertain the purity of the peak in the test sample, its insitu reflectance spectrum was compared with that of respective standards such as GA, EA, CR and GYA and found to be superimposable, thus confirming the peak purity. The contents of GA, EA, CR and GYA in the formulation were determined from the respective calibration plots. The GA, EA, CR and GYA contents in sugnil were found to be 1.38 %w/w, 0.52 %w/w, 0.31 %w/w and 0.176 %w/w respectively (Table 4). The dried TLC plates were photographed at 254 nm and 366 nm and the typical photographs are shown in figure 3a – 3e. Multiple bands are seen in the photo document 3a and each band corresponds to a ingredient in the formulation. In the photo documents 3b - 3e, the bands correspond to GA (brown spot), EA (gray spot), CR (green spot) and GYA (black spot) indicating the presence in sugnil.
**Fig-1b: HPTLC chromatogram of sugnil methanolic extract**  
[Peak 7 represents gallic acid (GA)]

**Fig-1c: HPTLC chromatogram of sugnil methanolic extract**  
[Peak 5 represents ellagic acid (EA)]

**Fig-1d: HPTLC chromatogram of sugnil methanolic extract**  
[Peak 6 represents curcuminoid (CR)]

**Fig-1e: HPTLC chromatogram of sugnil methanolic extract**  
[Peak 7 represents gymnemic acid (GYA)]
Fig-2a: HPTLC chromatogram of standard gallic acid (GA)

Fig-2b: HPTLC chromatogram of standard ellagic acid (EA)

Fig-2c: HPTLC chromatogram of standard curcuminoid (CR)

Fig-2d: HPTLC chromatogram of standard gymnemic acid (GYA)

[Peak 3 represents curcuminoid (CR)]
Table 4: Contents of marker compounds in *Sugnil*

<table>
<thead>
<tr>
<th>Marker compounds</th>
<th><em>Sugnil</em> (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic Acid</td>
<td>1.38</td>
</tr>
<tr>
<td>Ellagic Acid</td>
<td>0.52</td>
</tr>
<tr>
<td>Curcuminoid</td>
<td>0.31</td>
</tr>
<tr>
<td>Gymnemic acid</td>
<td>0.17</td>
</tr>
</tbody>
</table>

The presence of these bioactive compounds are reported to have several health benefits. Gallic acid, an important bioactive compound of *Eugenia jambolana* and triphala, has been shown to possess antiallergic, antimutagenic, anti-inflammatory and anticarcinogenic agent and strong natural antioxidant properties [2]. Ellagic acid, an important fraction of *Eugenia jambolana*, has been found to have anticarcinogenic [3], antifibrotic [4] and antioxidative [5] properties. Curcuminoid, an active fraction of *Curcuma longa* exhibits anti-inflammatory [6], antioxidant [7], anticarcinogenic [8] antiviral [9] and antimicrobial activity [10,11]. Beside these, curcumin has a variety of potentially therapeutic properties, such as antineoplastic, antiapoptotic, antiangiogenic, cytotoxic, immunomodulatory, [12] and antithrombotic, wound healing, antidiabetogenic, antistressor and antithrombogenic actions [13]. Gymnemic acid, the active fraction of *Gymnema sylvestre*, exhibits antidiabetic, antisweetener and anti-inflammatory activities. The possible mechanisms by which the gymnemic acids exert its hypoglycemic effects are: 1) it promotes regeneration of islet cells and increases secretion of insulin 2) it increases utilization of glucose by increasing the activities of enzymes responsible for utilization of glucose by insulin-dependant pathways but decreases gluconeogenic enzymes and sorbitol dehydrogenase, and 3) it causes inhibition of glucose absorption from intestine [14].

Overall, the data of HPTLC fingerprint indicates that *sugnil* was prepared from genuine plants or parts of the plants. It also resolves and quantifies major bioactive compounds effectively and assures the genuineness of the formulation.

**Alpha-glucosidase inhibitory action of *Sugnil***

One therapeutic approach for treating diabetes mellitus is to decrease postprandial hyperglycemia. This can be achieved by the suppression of carbohydrate hydrolyzing enzyme α-glucosidase. It is a membrane – bound enzyme located at the epithelium of the small intestine, hydrolyzes di- and oligosaccharides to glucose. Inhibition of activity of this enzyme reduces the rate of digestion of starch and results in a decrease in post-prandial blood glucose levels in diabetic patients [15]. Miglitol and acarbose are two commercially available synthetic drugs which inhibit the activity of α-glucosidase. There are a number of medicinal plants have been shown to suppress α-glucosidase activity and thereby exerts antidiabetic property [16]. Hence, this enzyme is considered to be one of the valuable therapeutic targets for diabetes treatment. Therefore, *sugnil* was assessed for *in vitro* α-glucosidase inhibitory activity.
The results revealed that water extract of *sugnil* efficiently inhibits α-glucosidase enzyme *in vitro* (Table 5). The percentage inhibition at 1.5 - 1000 µg/ml concentrations of drug showed a concentration-dependent increase in percentage inhibition. The percentage inhibition varied from 32.7±0.6 to 79.7±1.4 for highest concentration to the lowest concentration. The IC$_{50}$ (concentration with 50% inhibition) value of *sugnil* against α-glucosidase was found to be 30.1±0.53 (Table 6). The inhibitory activity of *sugnil* against α-glucosidase could be caused by the presence of bioactive phytoconstituents like flavonoids, tannins and phenols. These phytochemicals were found to possess α-glucosidase inhibitory activity [17,18]. The strong α-glucosidase inhibitory activity of *sugnil* substantiates its potent *in vitro* antidiabetic activity.

### Table 5: Alpha glucosidase inhibitory activity of SUGNIL

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th><em>Sugnil</em> (% inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>32.69±0.63</td>
</tr>
<tr>
<td>3</td>
<td>47.20±2.26</td>
</tr>
<tr>
<td>7</td>
<td>48.95±1.29</td>
</tr>
<tr>
<td>15</td>
<td>53.25±0.78</td>
</tr>
<tr>
<td>30</td>
<td>55.24±0.17</td>
</tr>
<tr>
<td>60</td>
<td>55.94±1.16</td>
</tr>
<tr>
<td>125</td>
<td>70.42±0.02</td>
</tr>
<tr>
<td>250</td>
<td>74.82±0.15</td>
</tr>
<tr>
<td>500</td>
<td>78.92±0.50</td>
</tr>
<tr>
<td>1000</td>
<td>79.71±1.37</td>
</tr>
</tbody>
</table>

### Table 6: IC$_{50}$ values of *Sugnil* and standard Meglitabose

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC$_{50}$ values (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sugnil</em> (water extract)</td>
<td>30.1±0.53</td>
</tr>
<tr>
<td>Meglitabose (standard)</td>
<td>30.26±4.01</td>
</tr>
</tbody>
</table>

**CONCLUSIONS**

The following conclusions were drawn after *in vitro* analysis of *sugnil*. Physiochemical analysis gives surety about the product which is genuinely prepared. Microbial screening shows that there was no microbial contamination in the product and thus, assures its quality and presumed safety. Nutritional assessment indicates its good nutritional value. Secondary metabolite analysis reveals the presence of active phytochemicals which are mainly involved in the pharmacological actions of *sugnil*. Heavy metal analysis confirms its non-toxicity during the course of treatment. HPTLC finger print indicates that the product *sugnil* was prepared from genuine plants or parts of the plants and presence of major bioactive compounds assures the genuineness of the formulation. The strong α-glucosidase inhibitory activity of *sugnil* substantiates its potent *in vitro* antidiabetic activity. These results show that the traditional Siddha antidiabetic polyherbal formulation *sugnil* is genuine and a standardized drug.
Fig-3a: TLC fingerprint profile of *sugnil* at 254 and 366 nm

Track I - *Sugnil* (5µl)
Track II - *Sugnil* (10µl)
Solvent - nHex:EA:FA:GAA (15:6:0.5:1.5)

Fig-3b: TLC fingerprint of *sugnil* with gallic acid standard at 254 nm

Track I - *Sugnil*
Track II - Gallic acid
Solvent - Tol:EA:FA:M (3:3:0.8:0.2)
Fig-3c: TLC fingerprint of sugnil with ellagic acid (EA) standard at 254nm

Fig-3d: TLC fingerprint of sugnil with Curcuminoid (CR) standard at 366 nm

Fig-3e: TLC fingerprint of sugnil with gymnemic acid standard at 254 nm

Track I - Sugnil
Track II - Ellagic acid
Solvent - Tol:EA:FA:MeOH (3:3:0.8:0.2)

Track I - Sugnil
Track II - Curcuminoid
Solvent - CHCl₃: Ethanol: AA (95:5:1)

Track I - Sugnil
Track II - Gymnemic acid
Solvent - nHex: EA: AA (33:14:5)
REFERENCES