ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

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

Paranthaman Karthikeyan[1*], Gopal Sathiya[1] and Subbramanium Sridhar[2]

[1]Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalainagar–608 002, Tamil Nadu, India.
[2] Ashram 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

ISSN: 0975-8585

INTRODUCTION

Popularity and increasing demand of herbal medicines create 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 Ayurverda 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 filted 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 50^0C, 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 440^0C 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 H_2O_2. 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, choloroform 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 F_{254} 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

Sample	-	Polyherbal Formulation *sugnil*
Sample prepared in	-	Methanol
Stationary phase	-	Silica gel GF_{254}
Mobile phase	-	nHex:EA:FA:GAA (15:6:0.5:1.5)
Scanning wavelength	-	300 nm
Sample concentration	-	10mg/ml
Applied volume	-	Track 1(5µl) and Track 2 (10µl)
Development mode	-	Ascending mode

Gallic Acid

Sample	-	Polyherbal Formulation *sugnil*
Standard	-	Gallic Acid (GA)
Sample prepared in	-	Methanol
Stationary phase	-	Slica gel GF_{254}
Mobile phase	-	Tol:EA:FA:M (3:3:0.8:0.2)
Scanning wavelength	-	280nm
Sample concentration	-	*Sugnil* (I-10mg/ml), GA (II-250 µg/ml)
Applied volume	-	Track 1(5 µl) anf Track 2 (2.5 µl)
Development mode	-	Ascending mode
Lamp	-	Deuterium

Ellagic Acid

Sample	-	Polyherbal Formulation *sugnil*
Standard	-	Ellagic Acid (EA)

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 105^0C 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 37^0C. The reaction was initiated by adding 1 ml of α-glucosidase enzyme (1U/ml) followed by incubation at 37^0C for 30 mins. Then, the mixture was heated in boiling water bath to stop the reaction. The amount of librated 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.

ISSN: 0975-8585

Table 1: Physicochemical and Phytochemical analysis of *Sugnil*
(Values are mean of three determinations ± SEM)

Physio-chemical analysis		Phytochemical analysis			
Ash content	*Sugnil* (%w/w)	Primary metabolites	*Sugnil* (mg/g)	Secondary metabolites	*Sugnil* (% w/w)
Total ash	13.55	Carbohydrates	105	Phenols	17.74
Acid soluble	6.85	Steroids	78	Tannins	16.54
Water soluble	6.85	Proteins	28	Flavonoids	11.09

Table 2: Microbial Analysis of *Sugnil*

S.No	Micro organisms	Absent/Present
1.	*E. coli*	Absent
2	*S.Aureus*	Absent
3	*P. Aeraginosa*	Absent
4	*Salmonella*	Absent
5	TAMC	620 Cfu/gm
6	TYMC	93 Cfu/gm

TAMC – Total areobic 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*

Sample	Elements									
	Fe (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)	Cr (ppm)	Pb (ppm)	As (ppm)	Hg (ppm)	Se (ppm)	Co (ppm)
Sugnil	0.885	0.018	1.977	0.129	0.015	0.068	ND	1.344	ND	0.042

*ND – Not detected

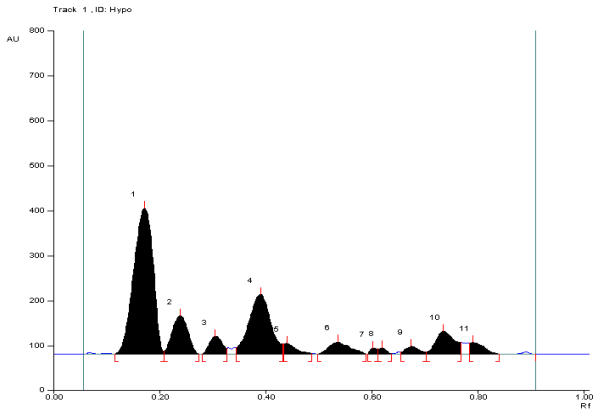
ISSN: 0975-8585

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-1a: HPTLC Finger print of *Sugnil*



ISSN: 0975-8585

Fig-1b: HPTLC chromatogram of *sugnil* methanolic extract [Peak 7 represents gallic acid (GA)]

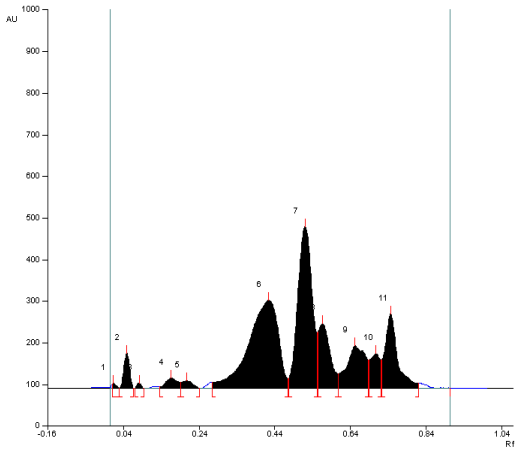


Fig-1c: HPTLC chromatogram of *sugnil* methanolic extract [Peak 5 represents ellagic acid (EA)]

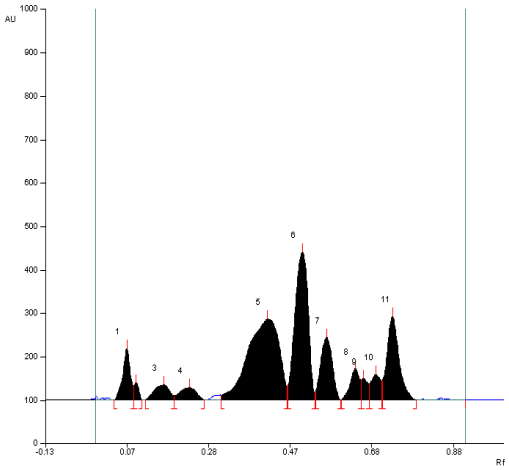


Fig-1d: HPTLC chromatogram of *sugnil* methanolic extract [Peak 6 represents curcuminoid (CR)]

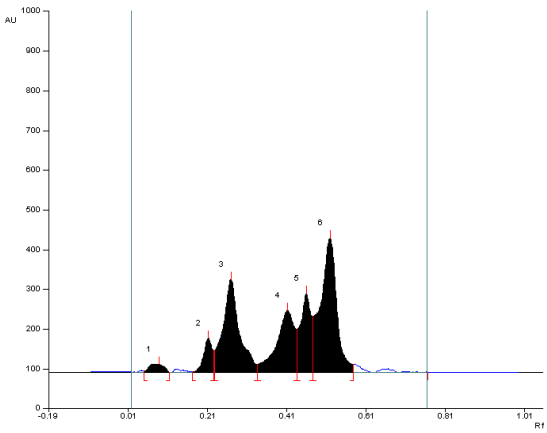
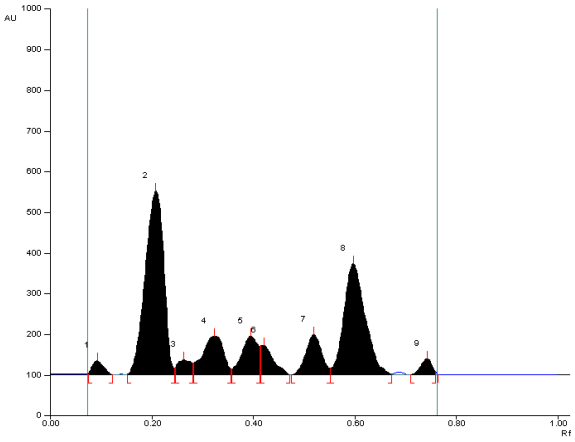


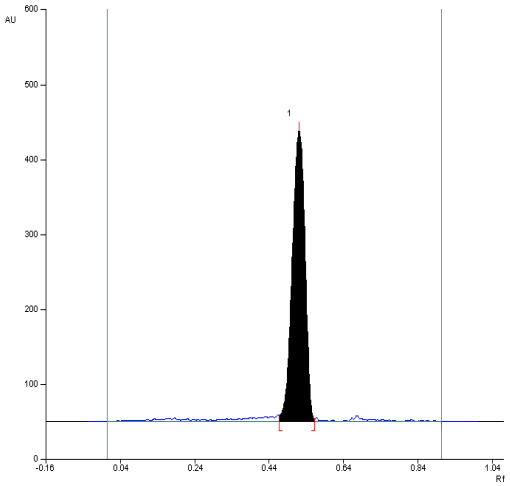
Fig-1e: HPTLC chromatogram of *sugnil* methanolic extract [Peak 7 represents gymnemic acid (GYA)]



ISSN: 0975-8585

Fig-2a: HPTLC chromatogram of standard gallic acid (GA)

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



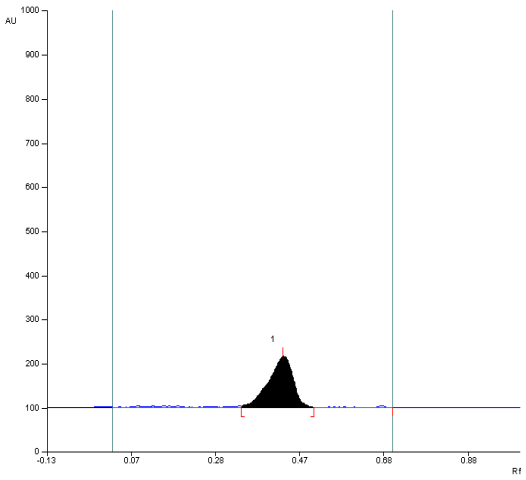
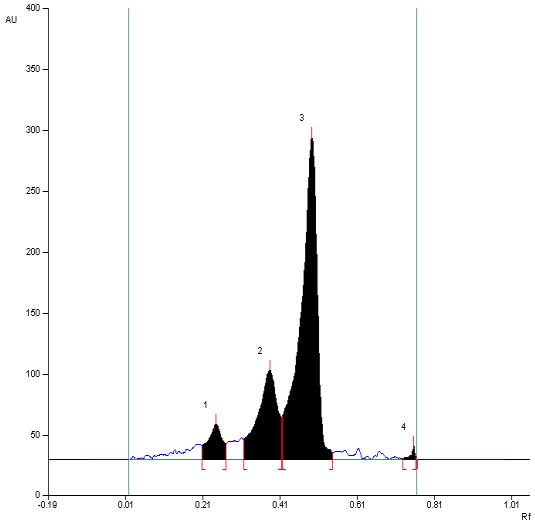
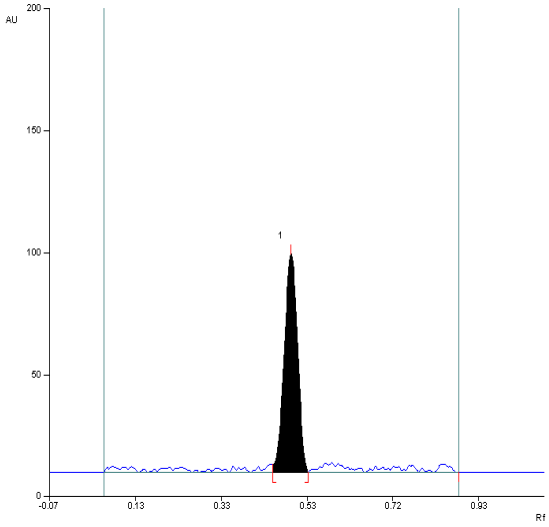


Fig-2c: HPTLC chromatogram of standard curcuminoid (CR)

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

[Peak 3 represents curcuminoid (CR)]





ISSN: 0975-8585

Table 4: Contents of marker compounds in *Sugnil*

Marker compounds	*Sugnil* (% w/w)
Gallic Acid	1.38
Ellagic Acid	0.52
Curcuminoid	0.31
Gymnemic acid	0.17

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 antiinflammatory [6], antioxidant [7], anticarcinogenic [8] antiviral [9] and antimicrobial activitity [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 antilithogenic 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 finger print 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 genuiness 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 *invitro* α-glucosidase inhibitory activity.

The results revealed that water extract of *sugnil* efficiently inhibits α-glucosidase enzyme *invitro* (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 *invitro* antidiabetic activity.

Table 5: Alpha glucosidase inhibitory activity of SUGNIL

Concentration (ug/ml)	*Sugnil* (% inhibition)
1.5	32.69±0.63
3	47.20±2.26
7	48.95±1.29
15	53.25±0.78
30	55.24±0.17
60	55.94±1.16
125	70.42±0.02
250	74.82±0.15
500	78.92±0.50
1000	79.71±1.37

Table 6: IC_{50} values of *Sugnil* and standard Meglitabose

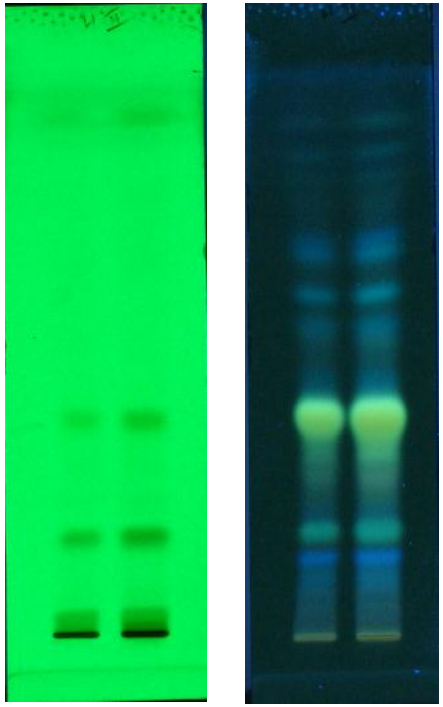
Sample	IC_{50} values (mg/ml)
	α- glucosidase enzyme
Sugnil (water extract)	30.1±0.53
Meglitabose (standard)	30.26±4.01

CONCLUSIONS

The following conclusions were drawn after *invitro* 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 genuiness of the formulation. The strong α-glucosidase inhibitory activity of *sugnil* substantiates its potent *invitro* antidiabetic activity. These results show that the traditional Siddha antidiabetic polyherbal formulation *sugnil* is genuine and a standardized drug.

ISSN: 0975-8585

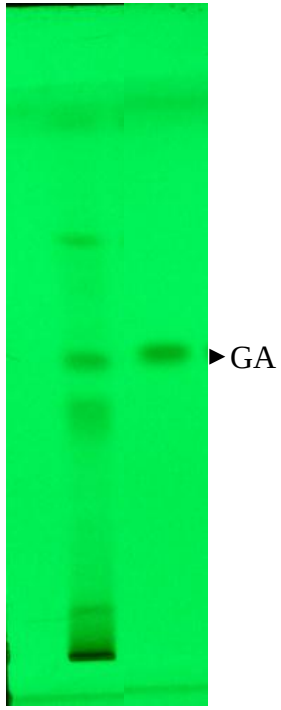
Fig-3a: TLC fingerprint profile of *sugnil* at 254 and 366 nm



I II I II

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

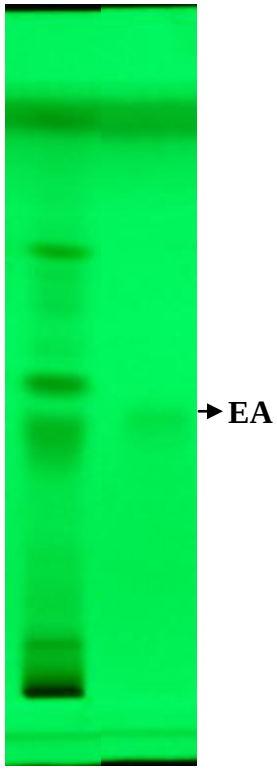


I II

Track I - *Sugnil*
Track II - Galic acid
Solvent - Tol:EA:FA:M (3:3:0.8:0.2)

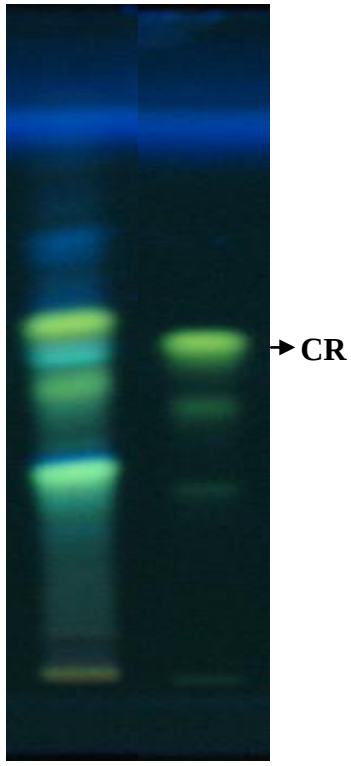
ISSN: 0975-8585

Fig-3c: TLC fingerprint of *sugnil* with ellagic acid (EA) standard at 254nm



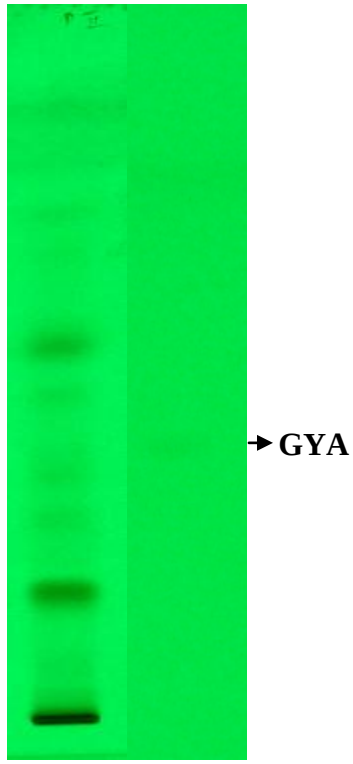
Track I - *Sugnil*
Track II - Ellagic acid
Solvent - Tol:EA:FA:MeOH (3:3:0.8:0.2)

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



Track I - *Sugnil*
Track II - Curcuminoid
Solvent - $CHCl_3$: Ethanol: AA (95:5:1)

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



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

ISSN: 0975-8585

REFERENCES

[1] Caudhury RR. Herbal medicines for human health. World Health Organization, New Delhi, India, 1992, 400.
[2] Jadon A, Bhadauria M, Shukla S . J. Ethnopharmacol 2007; 109: 214–218.
[3] Niho N, Shibutani M, Tamura T, Toyoda K, Uneyama C, Takahashi N, Hirose M. Food Chem Toxicol 2001; 39: 1063–1070.
[4] Mukhtar H, Das M, Bickers DR. Cancer Res 1986; 46: 2262-5.
[5] Thresiamma KC, Kuttan R. Indian J Physiol Pharmacol 1996; 40: 363–366.
[6] Osawa T, Ide A, Jeng DS, Namiki M. Food Chem 1987; 35: 808–812
[7] Chainani-Wu N. J. Altern. Complement Med 2003; 9: 161-8.
[8] Lantz RC, Chen GJ, Solyom AM, Jolad SD, Timmermann B. Phytomedicine 2005; 12: 445-452.
[9] Frank N, Knauft J, Amelung F, Nair J, Wesch H, Bartsch H. Mutation Res/Fundamental and Mol Mech of Mutagenesis 2003; 523-524: 127–135.
[10] Suai Z, Salto R, Li J, Craik C, Montellano PRC. Bioorg and Medicinal Chem 1993; 1: 415-422.
[11] Mahady, GB, Pendland SL, Yun G, Lu ZZ. Anticancer Res 2002; 22: 4179-81.
[12] Han S, Yang Y. Dyes and Pigments 2005; 64: 157-161.
[13] Strimpakos AS, Sharma RA. Antioxid Redox Signal 2008; 10(3): 511-45.
[14] Kanetkar PV, Laddha KS, Kamat MY. Poster presented at the 16th ICFOST meet organized by CFTRI and DFRL. Mysore, India: 2004. Gymnemic acids: A molecular perspective of its action on carbohydrate metabolism.
[15] Karthic K, Kirthiram KS, Sadasivam S, Thayumanavan B. Ind J Exp Bio 2008; 46: 677-680.
[16] Pranav K, Prabhakar, Doble MA. Curr Dia Rev 2008; 4: 291-308.
[17] Haraguchi H, Ohmi I, Sakai S, Ukuda AF. J Nat Prod 1996; 59: 443-445.
[18] Lee HS, and Kim MK. Food sci. Biotech 2001; 10: 172-177.