

Research Article

In Vitro Investigation of Antidiabetic Potential of Selected Traditional Medicinal Plants

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ABSTRACT

Diabetes Mellitus is a systemic metabolic disease characterized by hyperglycemia, abnormal elevated levels of lipid, and fat in blood and hypoinsulinaemia. This study aims at determining the antidiabetic potential of five traditional medicinal plants such as, *Sarcostemma brevistigma*, *Grewia hirsuta*, *Indigofera aspalathoides*, *Memecylon edule* and *Solanum trilobatum*. The selected plant parts were subjected to solvent extraction and analyzed for their antidiabetic activity using *in vitro* assays such as, α -amylase inhibition assay, glucose diffusion assay, glucose uptake by yeast cells, and nonenzymatic glycosylation assay followed by phytochemical analysis and thin layer chromatography. The results of the assays suggest that among the selected plants, methanol extract of the *S. brevistigma* was significant in inhibiting the activity of α -amylase with IC₅₀ value of 250 μ g/ml. Further assays proved that the same was efficient in its antidiabetic potential. The phytochemical analysis showed the presence of phenols, flavonoids, and alkaloids in major amount. In addition, the thin layer chromatography revealed that five distinct compounds were present in the methanol extract of *S. brevistigma*, which might be responsible for its antidiabetic activity. Thus the study suggests that *Sarcostemma brevistigma* might be considered as a potential source of natural antidiabetic agents.

Keywords: *Sarcostemma brevistigma*, α -amylase, glucose uptake, glucose diffusion, glycosylation, TLC, antidiabetic potential.

INTRODUCTION

Diabetes Mellitus (DM) is a systemic metabolic disease characterized by hyperglycemia, abnormal elevated levels of lipid, and fat in blood and hypoinsulinaemia (Rao *et al.*, 2010). According to WHO, the global prevalence of diabetes is estimated to increase from 4% in 1995 to 5.4% by the year 2025 majorly in the developing countries (Jayaprasad *et al.*, 2011). India, presently, has the largest number of diabetic patients in the world and has been infamously known as the “diabetic capital of the world” (Abate *et al.*, 2007). The classical symptoms of type 1 diabetes are polyuria (frequent urination), polydipsia (increased thirst), polyphagia (increased hunger), and weight loss (Plevyak, 2011).

In recent years, drug therapies have been in use for the treatment of diabetes. Some of the standard synthetic drugs used for the treatment of diabetes are sulfonylureas, biguanides, α -glucosidase inhibitors, glinides, etc. These drugs tend to cause side effects like nausea, vomiting, abdominal pain, diarrhoea, head ache, abnormal weight gain, allergic reaction, low blood glucose, dark urine, fluid retention, or swelling. Moreover, they are not safe for use during pregnancy (Anbu *et al.*, 2013). Active research has been performed on traditionally available medicinal plants for the discovery of new antidiabetic drug as an alternative

for synthetic drugs. Hence the current study is focussed to evaluate the antidiabetic potential of selected medicinal plants.

MATERIALS AND METHODS

Plant Collection and Direct Extraction: The fresh leaves of plants *Sarcostemma brevistigma*, *Grewia hirsuta*, *Memecylon edule*, *Gmelina asiatica*, and *Solanum trilobatum* were collected from Melachery forest, Gingee, Tamil Nadu, and were authenticated by Dr. Mathivanan, CAS in Botany, Maraimalai campus, Chennai. The collected plant leaves were shade dried for 2 weeks, coarsely powdered, and subjected to direct extraction.

Extraction of plant material: Coarsely powdered leaves were subjected to direct extraction using solvents of varying polarity such as hexane, ethyl acetate, and methanol by following the method of Eloff (1998). 10g of each leaf powders was immersed in 100ml of respective solvents (1:10 w/v) and kept under shaking condition for 24hrs with intermittent filtration. The filtrates were collected and condensed to obtain the crude extract.

Evaluation of Antidiabetic Potential

α -Amylase Inhibition: In α -amylase inhibition method, the enzyme solution was prepared by dissolving α -amylase in 20mM phosphate buffer (6.9) at the

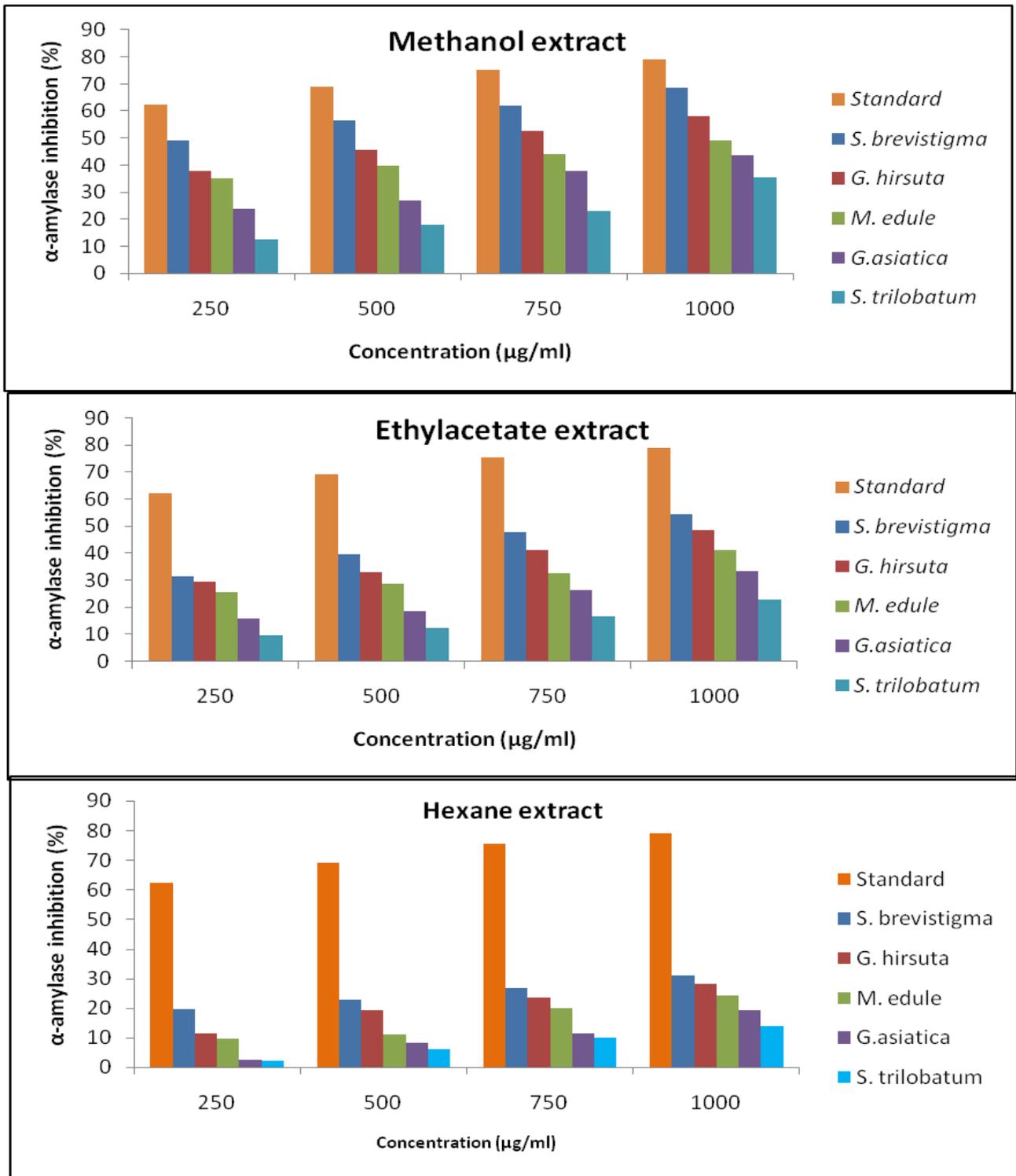


Fig. 1 Inhibitory effects of extracts of the selected plants on α -amylase activity

concentration of 0.5mg/ml. 1ml of the extract of various concentrations (250, 500, 750, 1000 µg/ml) and 1ml of enzyme solutions were mixed together and incubated at 25°C for 10min. After incubation, 1ml of starch (0.5%) solution was added to the mixture and further incubated at 25°C for 10min. The reaction was then stopped by adding 2ml of dinitrosalicylic acid (DNS, color reagent), heating the reaction mixture in a boiling water bath (5min). After cooling, the absorbance was measured colorimetrically at

565 nm (Rammohan *et al.*, 2008). The inhibition percentage was calculated using the given formula, %inhibition =

$$\frac{\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}}{\text{Abs}_{\text{Control}}} \times 100$$

where Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample

Nonenzymatic glycosylation of haemoglobin method: Glucose (2%), haemoglobin (0.06%), and Gentamycin

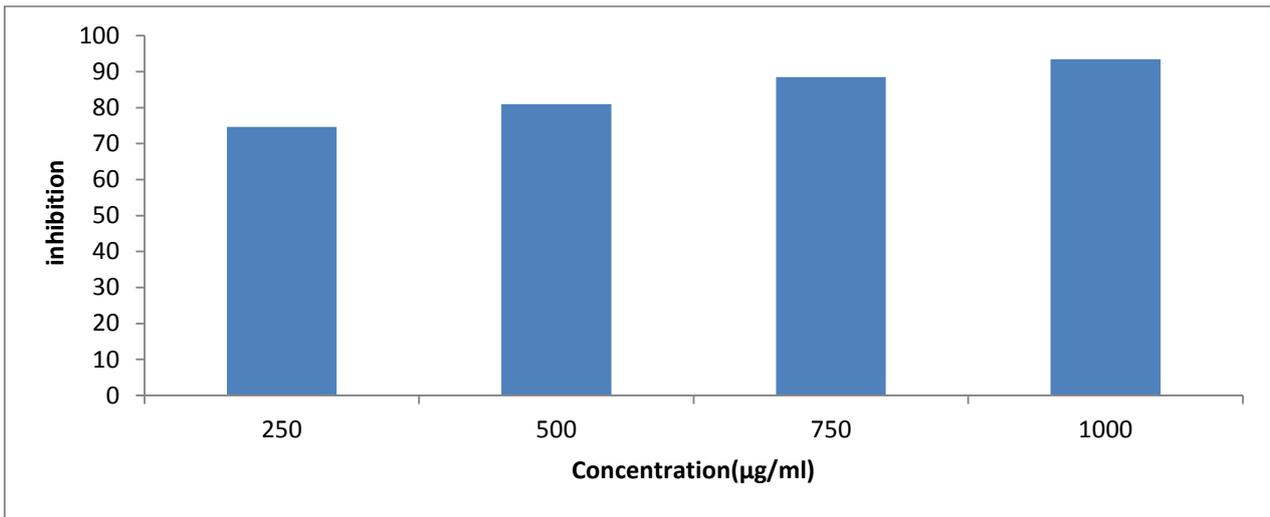


Fig. 2 Effect of methanol extract of *S. brevistigma* on glycosylation of hemoglobin

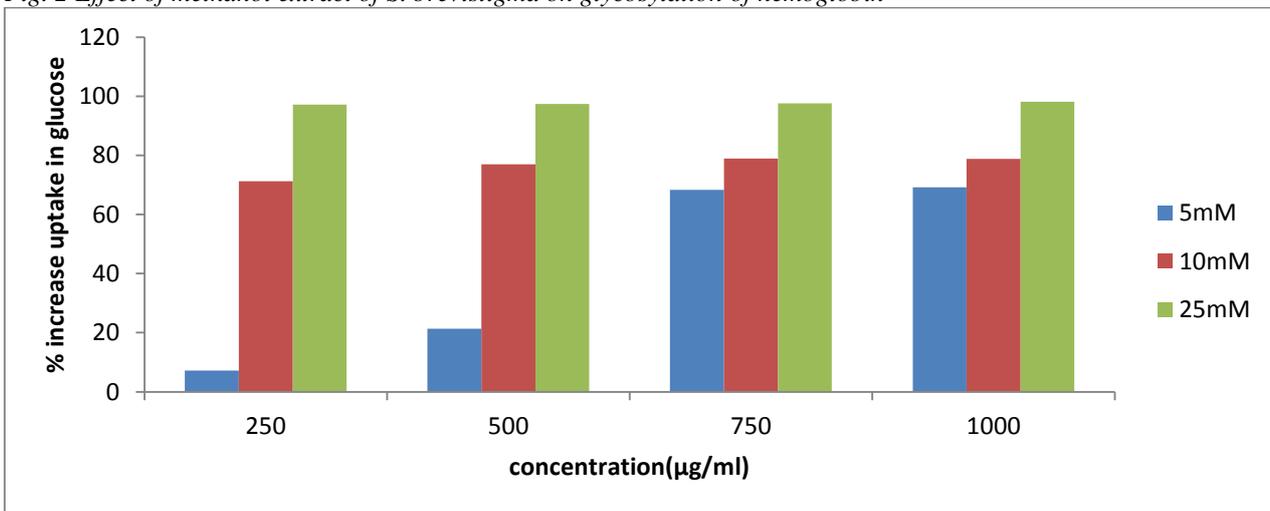


Fig. 3 Effect of methanol extract of *S. brevistigma* on glucose uptake by yeast cells

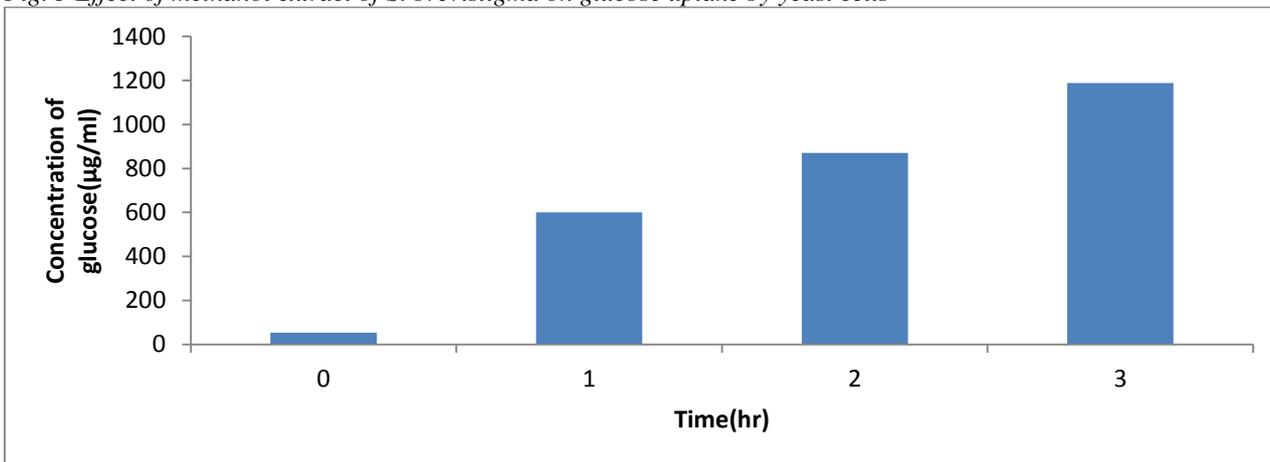


Fig. 4 Effect of methanol extract of *S. brevistigma* on glucose diffusion

(0.02%) solutions were prepared in phosphate buffer 0.01 M, pH 7.4. 1 ml each of the above solutions was mixed and 1ml of the methanol extract of varying concentrations was added to it, respectively. The reaction mixture was incubated in dark at room temperature for 72hrs and then the degree of glycosylation of haemoglobin was measured colorimetrically at 520nm (Chandrasekar *et al.*, 2012). Metformin was used as a standard drug for the assay and percentage inhibition was calculated using the formula,

$$\% \text{inhibition} = \frac{\text{Absorbance}_{\text{Sample}} - \text{Absorbance}_{\text{Control}}}{\text{Absorbance}_{\text{Sample}}} \times 100$$

where Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample

Glucose Uptake by Yeast Cells: Yeast suspension was prepared by repeated washing (by centrifugation 3,000×g; 5 min) in distilled water until the supernatant fluids were

Table 1: Qualitative phytochemical analysis of the methanol extract of *S. brevistigma*

S.no	Phyto chemical	Result
1	Alkaloid	++
2	Flavonoid	++
3	Phenols	+++
4	Saponin	+
5	Carbohydrate	+
6	Protein/amino acids	-

Table 2: Quantitative phytochemical analysis of the methanol extract of *S. brevistigma*

S.no	Phyto chemical	Amount
1	Total alkaloids	0.03g/g
2	Total polyphenols	1911.9 GAE/g
3	Total flavonoids	1111.48 QE /g

clear. A 10% (v/v) suspension was prepared with the supernatant fluid. 1mL of the glucose solution (5, 10, and 25 mM) was added to various concentrations of methanol extract (250, 500, 750, and 1000 µg) and incubated for 10 min at 37 °C. Reaction was started by adding 100 µl of yeast suspension, vortexed and further incubated at 37 °C for 60 min. After 60 min, the reaction mixture was centrifuged (2,500×g, 5 min) and the glucose content was estimated in the supernatant. Metronidazole was taken as a standard drug. The percentage increase in glucose uptake by yeast cells was calculated using the following formula: %inhibition =

$$\frac{\text{Absorbance}_{\text{Sample}} - \text{Absorbance}_{\text{Control}}}{\text{Absorbance}_{\text{Sample}}} \times 100$$

Where Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample.

Glucose Diffusion Assay: This assay was performed as described by Gallagher *et al.*, (2013) with minor modifications. 2 ml of 0.15 M NaCl containing 0.22mM D-glucose was loaded into a dialysis tube containing plant extract (50g/L) and the dialysis tube was sealed. The sealed tube was then placed in a centrifuge tube containing 45 ml of 0.15 M NaCl and kept in an orbital shaker at a room temperature. The diffusion of glucose into the external solution was monitored by measuring the glucose concentration in the external solution every 60min.

Qualitative Phytochemical Analysis: The methanol extract of *S. brevistigma* was subjected to various biochemical tests to screen the presence of phytochemicals such as, alkaloids, flavonoids, phenols, saponins, tannins, carbohydrates, and glycosides by following the methods of Harborne (1998).

Quantitative Phytochemical Estimation

Determination of total phenols: The total phenolics contents in different solvent extracts were determined using Folin–Ciocalteu's reagent (FCR) following the assay of Damodar (2011) with small modifications. 0.5 ml of the extract was mixed with 0.1ml FCR (diluted 1:2 v/v), incubated for 15min, followed by the addition of 2.5 ml of saturated sodium carbonate solution. The final volume of the tubes was made up to 10 ml with distilled water, allowed to stand for 30 min at room temperature and the absorbance was measured at 750nm. A calibration curve

was constructed using gallic acid solution as a standard and total phenolic content of the extract was expressed as a Gallic Acid Equivalents/g sample.

Estimation of Total Flavonoids: Total flavonoid content was determined by Aluminium chloride method using Quercetin as a standard. 1ml of the methanol extract of *S. brevistigma* was added to 4 ml of distilled water and incubated for 5min. After incubation, 0.3 ml of NaNO₂ (5%) and 0.5 ml of AlCl₃ were added and the mixture was re-incubated at room temperature for 6 min followed by the addition of 0.5ml of 1M NaOH. The final volume was made up to 10ml with distilled water and the absorbance of the reaction mixture was measured at 510 nm (Badugu, 2012).

Estimation of Total Alkaloids: The total alkaloid content of *S. brevistigma* was estimated using the method of Harborne. 5 g of the sample (leaf powder) was weighed and added to 200 ml of acetic acid (10% in ethanol), covered and allowed to stand for 4h. The solution was then filtered and the filtrate was concentrated on a water bath to one-quarter of the original volume. To the concentrate, NH₄OH was added dropwise until the precipitation was complete. The whole solution was allowed to settle down and the precipitate was collected, washed with dilute ammonium hydroxide and filtered. The residue is the alkaloid, which was dried and weighed.

Thin Layer Chromatography: The methanol extract of *S. brevistigma* was further subjected to TLC to study its compound profile. The extract was spotted on precoated silica plates and developed with methanol: chloroform mixture in varying ratio. The run TLC plates were visualized under UV illumination and Iodine vapors. The ratio in which distinct bands appeared was optimized and R_f (Retention factor) values of the bands were calculated.

RESULTS AND DISCUSSION

Extracts of *S. brevistigma* were obtained using different solvents of varying polarity such as hexane, ethyl acetate, and methanol. These extracts were filtered, re-extracted with same solvents, respectively, condensed to dryness to obtain crude extracts.

α- Amylase Inhibition: Alpha amylase is an enzyme that hydrolyses alpha-bonds of alpha-linked polysaccharide such as starch to yield high levels of glucose and maltose. α-amylase inhibitors bind to α-bond of polysaccharide and prevent break down of polysaccharide into mono and disaccharide (Nair *et al.*, 2013). *In vitro* inhibitory assay of α-amylase was performed for all the solvent extracts of the selected plants. From the data obtained, it was found that methanol extract of *S. brevistigma* showed significant inhibitory activity when compared with all the other plant extracts (Fig. 1). The inhibition varied from 49 to 68% in the concentration range of 250 to 1000µg/ml. The results obtained clearly suggest that the methanol extract of *S. brevistigma* is capable of effectively inhibiting the α-amylase activity.

Nonenzymatic Glycosylation of Haemoglobin Method: The haemoglobin present in the red blood corpuscles has a tendency to get bound to glucose. The inhibitory activity

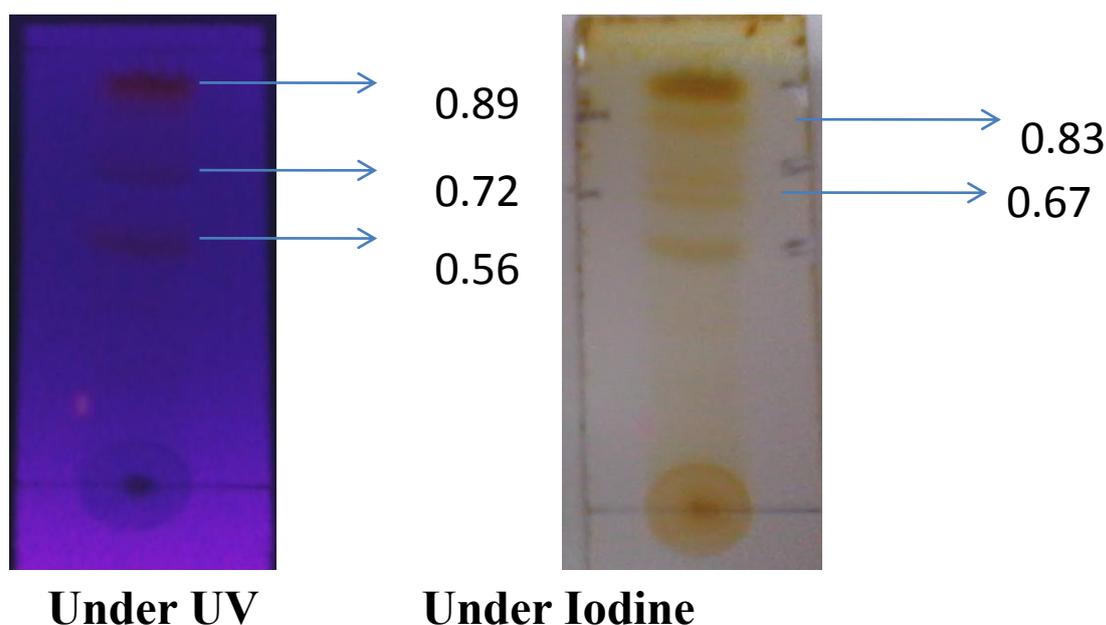


Fig. 5 Thin layer chromatogram of the methanol extract of S. brevistigma

of methanol extract of *S. brevistigma* was found and compared with the standard drug, acarbose. Results showed that the methanol extract showed higher inhibitory activity up to 93% (Fig 2). The greater the blood-glucose concentration, the greater is the amount of glucose-bound haemoglobin. As the concentration of the drug increases, formation of the glucose-haemoglobin complex decreases and free haemoglobin increases, which shows the inhibition of glycosylated haemoglobin (Acharya *et al.*, 1980).

Glucose Uptake by Yeast Cells: It was studied that the glucose uptake rate was increased with the increasing concentration of the plant extract and decreased with the increasing extracellular glucose concentration (Fig. 3). It is stated that the transport of glucose across yeast cell membrane occurs by facilitated diffusion down the concentration gradient. Hence glucose transport occurs only if the intracellular glucose is effectively reduced (utilized) (Ahmed *et al.*, 2009). The data obtained clearly suggests that the methanol extract of *S. brevistigma* is capable of effectively enhancing glucose uptake which in turn suggests that it is capable of enhancing the effective glucose utilization, thereby controlling blood glucose level.

Glucose Diffusion Assay: The level of inhibition of glucose movement by the plant extract at various intervals of time which was assayed and compared with the control in the absence of plant extract. Methanol extract of *S. brevistigma* significantly decreased the glucose movement across the membrane when compared to the control (Fig 4).

Qualitative and Quantitative Phytochemical Screening: Various chemical tests were performed using the standard procedures to identify the phytoconstituents. These screening tests revealed the presence of various plant constituents present in the plant. Among the various phytoconstituents, phenols, flavonoids, and alkaloids were majorly present (Table 1). These phytochemicals were

further quantified as 1911.9 GAE/g, 1111.48 QE/g and 0.03g/g, respectively (Table 2).

Thin Layer Chromatography: The chromatogram was developed using the solvent ratio (1:9), showed five distinct compounds. The compounds spotted under UV were found to have R_f values of 0.89, 0.72 and 0.56, respectively. Furthermore, two more compounds with R_f values 0.83 and 0.67 were spotted under iodine (Fig. 5).

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