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Research Article

Invitro Evaluation of Antidiabetic Potential and Phytochemical Profile of *Psoralea corylifolia* Seeds

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ABSTRACT
Diabetes is a metabolic disorder that occurs due to poor secretion of insulin by pancreas. Insulin helps to metabolize blood sugar. Hence this results in high glucose level in blood. Presently, diabetes is considered as a big killer and is among the most significant diseases in the developed world. The incidence of diabetes is increasing every day and this indicates the increasing need for the treatment of diabetes. The blood glucose level can be regulated by various mechanisms. *In vitro* antidiabetic assays such as glucose uptake by yeast cells, glucose diffusion assay, alpha amylase inhibition assay and glycosylation of hemoglobin assay are performed. Using these assays, the antidiabetic activity of the plant *Psoralea corylifolia* is determined. Qualitative and quantitative phytochemical analysis reveals all the bioactive compounds present in the crude extract of the plant. Our results show that methanolic extract has an effective activity when compared to ethyl acetate and hexane extracts. Hence methanolic extract of *Psoralea corylifolia* possess higher antidiabetic activity. The compounds which contribute to its antidiabetic activity were identified, characterized and separated by thin layer chromatography. MTT assay for L6 cell lines was performed and was found that the plant extract showed a minimum toxicity.

Keywords: Diabetes, glucose, extract, antidiabetic activity, *in vitro* assay, cell lines.

INTRODUCTION

Diabetes is a metabolic disorder with a disturbed carbohydrate metabolism due to any defect in insulin secretion, action or both (Report of a WHO consultation). About 2-3% of the world's population is affected by diabetes [1]. Diabetes is more common in the developed countries. It ranks among the top 5 significant diseases in the world [2]. Diabetes is more prevalent in aged people and in India it's the 7th leading disease. The causes of diabetes occur with two processes - metabolic process and auto immune process. Factors such as age, obesity, physical inactivity, excess eating may prevent the use of insulin by the body. This stops the metabolism of carbohydrates. Sometimes the body's immune system may demolish the beta cells of pancreas, thus leading to diabetes. The factors involved are genetics, family history and environmental factors[3]. The major symptoms are polydipsia, polyurea and polyphagia[4].

Nowadays many new drugs are discovered as treatment to diabetes. But, some risks are involved in the usage of these therapeutic agents. Glucosidase and lipase inhibitors, which have been used as medicines give rise to certain side effects. For instance, acarbose has a low efficacy in decreasing the glycemic levels. Lipase inhibitor produces a weight loss in patients and some others cause hepatotoxicity, abdominal pain, flatulence, diarrhoea and hypoglycaemia[5].

Medicinal plants can be used for the treatment of diabetes since they are devoid of side effects. WHO recommends the use of herbal medicines as a cure for diabetes [6]. Medicinal plants are safer, specific and effective compared to the modern medicines. Only some plants have been scientifically validated, while more than 800 plants with antidiabetic potential exist in the ayurvedic and Indian traditions [7].With this background information, the present study is aimed at investigating the antidiabetic potential of *Psoralea corylifolia*. *P. corylifolia* is a medicinally important plant in India. The seeds of this plant act as a laxative, aphrodisiac, anthelminitic, diuretic and diaphoretic. The seeds serve as a cure for leprosy, psoriasis, leucoderma and inflammation of skin. In addition, they can inhibit the growth of *Staphylococcus citreus*, *S. aurues* and *S. albus*. The seeds are commonly named as babchi seeds [8]. The seeds of *Psoralea corylifolia* were also proved to possess anti inflammatory activity. Oil from the seeds can be used in the treatment of vitiligo [9]. Phytochemical analysis demonstrated the presence of flavanoids, tannins and reducing sugars [10].

MATERIALS AND METHODS

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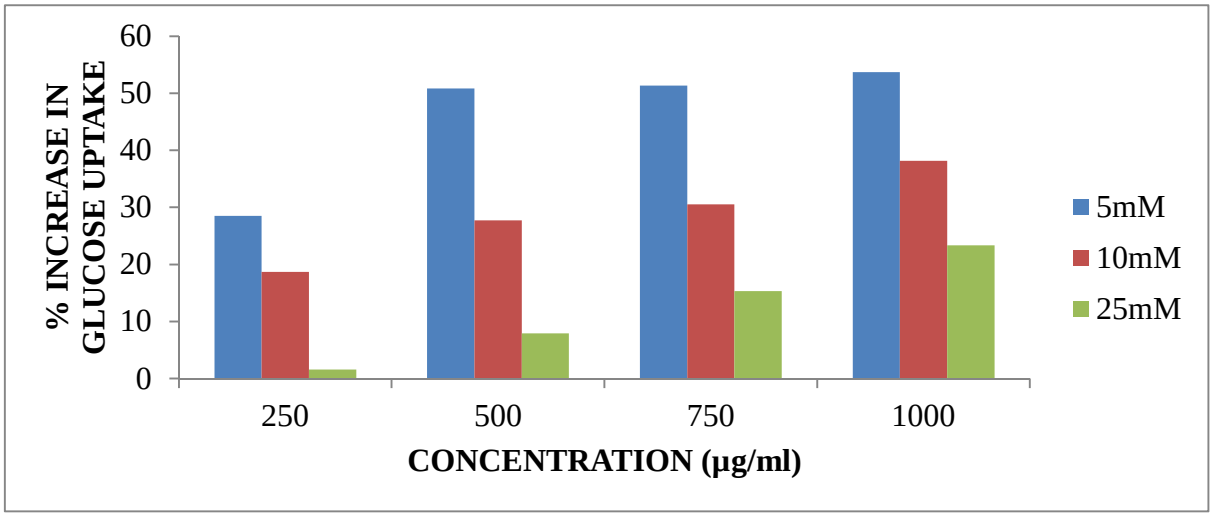


Fig. 1: Effect of methanol extract of Psoralea corylifolia on glucose uptake by yeast cells

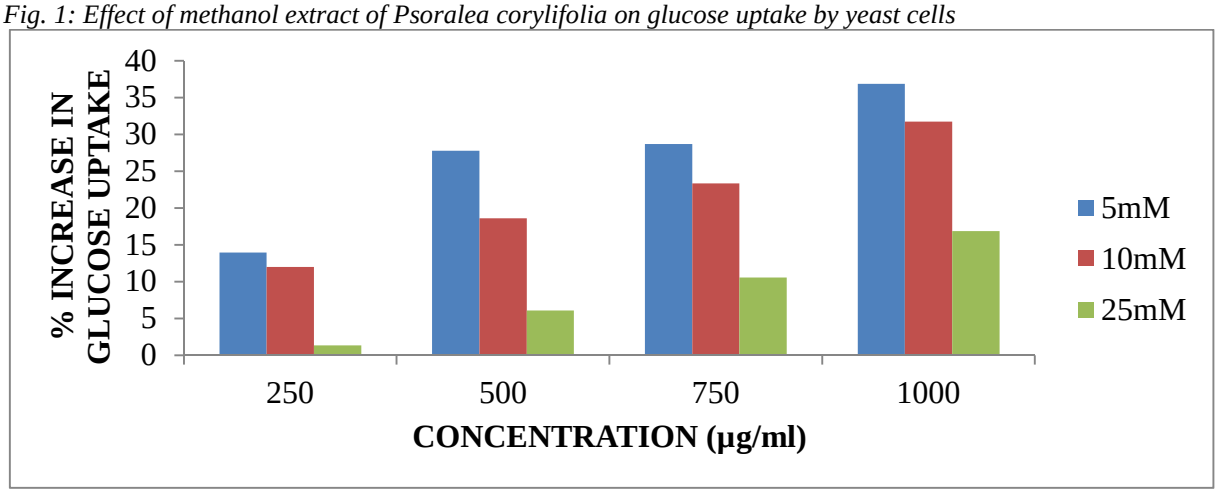


Fig. 2: Effect of ethyl acetate extract of Psoralea corylifolia on glucose uptake by yeast cells

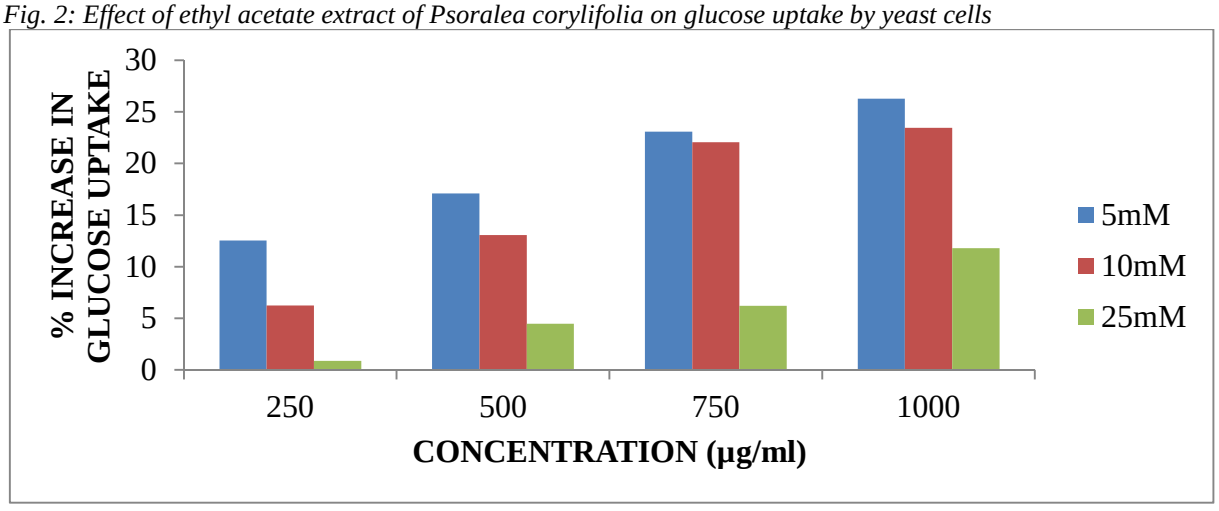


Fig. 3: Effect of hexane extract of Psoralea corylifolia on glucose uptake by yeast cells

Collection and extraction of Plant Sample: The seeds of the plant were collected from Koyambedu market, Chennai and cleaned by washing in water and air dried under shade. Dried sample was grounded into a coarse powder and subjected to direct extraction with chloroform, ethyl acetate and methanol in the ratio of 1:10 [11]. The solvent was removed by placing the extracts in distillation unit in the respective temperature (Eloff, 1998). The extracted residues were weighed and re-dissolved in different solvents to yield 10 mg/ml solutions ready for further analysis.

Evaluation of *invitro* Antidiabetic Potential of *Psoralea corylifolia*

Glucose Uptake by Yeast Cells: Commercial baker's yeast was thoroughly washed by repeated centrifugation at 3000rpm for 5mins. The supernatant was collected and 10ml of 10% (v/v) yeast cell suspension was prepared. To different concentrations of plant extract (250, 500, 750 and 1000µg/ml), 1ml of glucose solution at concentrations – 5mM, 10mM, 25mM were added and incubated at 37°C for 10mins. Reaction was started by adding 100µl of 10% yeast suspension to the above mixture and incubated at 37°C for 1hr. After incubation, the mixture was centrifuged at 2500rpm for 5mins. Glucose estimation was performed with the supernatant [12].

The percentage increase in glucose uptake =

$$\frac{Abs_{sample} - Abs_{control}}{Abs_{sample}} * 100$$

$Abs_{control}$ = Absorbance of the control; Abs_{sample} = Absorbance of the sample

Glucose Diffusion Assay: Glucose diffusion assay was performed by the method described by Edwards. A dialysis membrane (6cm*15mm) was sealed at one end and 1ml of sample (1ml of 50g/l plant extract in 1% carboxy methyl

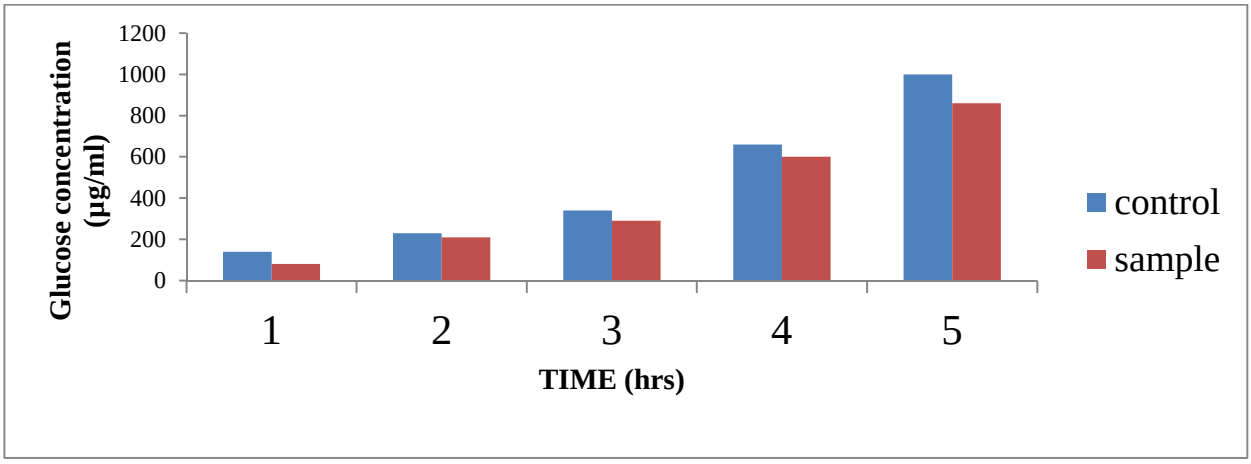


Fig. 4: Effect of methanol extract of Psoralea corylifolia on glucose diffusion

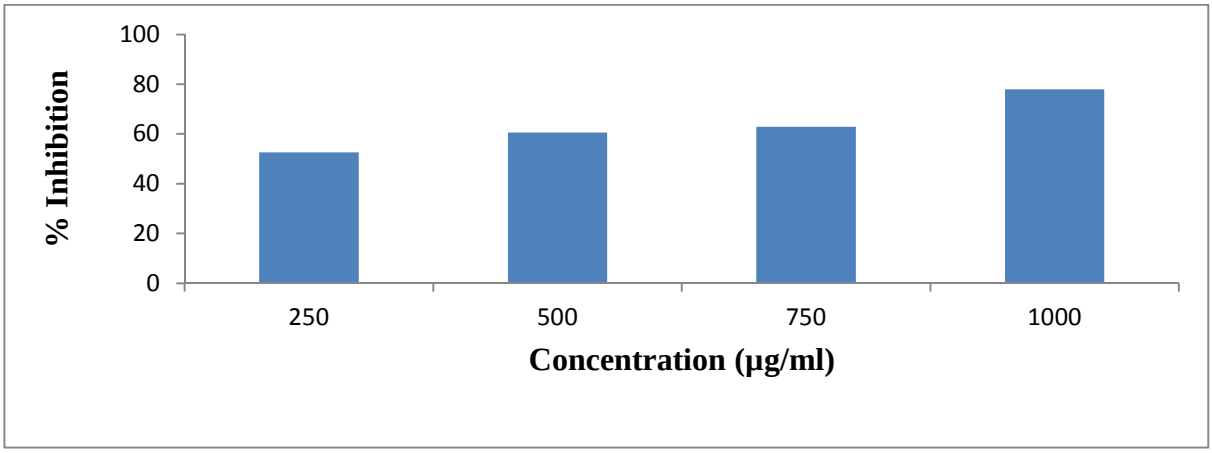


Fig. 5: Effect of methanol extract of Psoralea corylifolia on α amylase inhibition

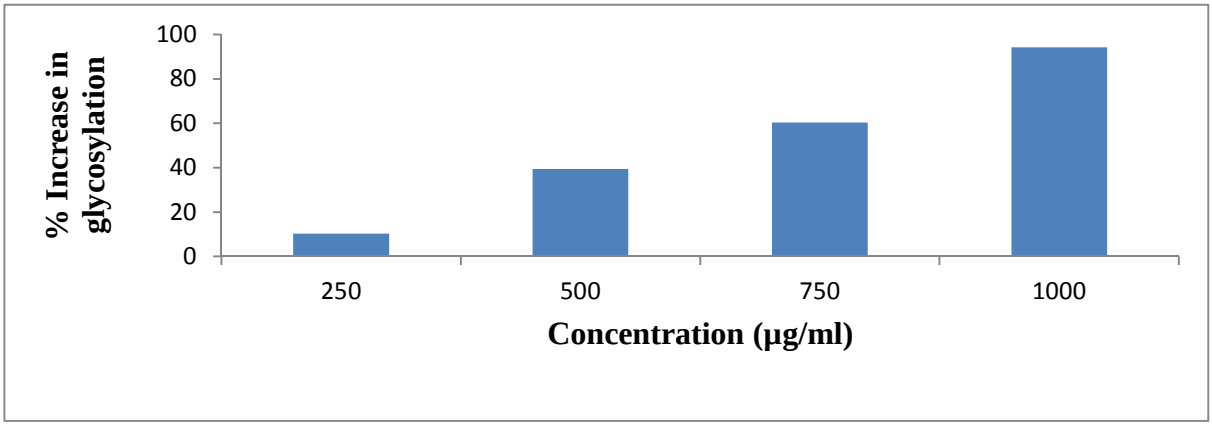
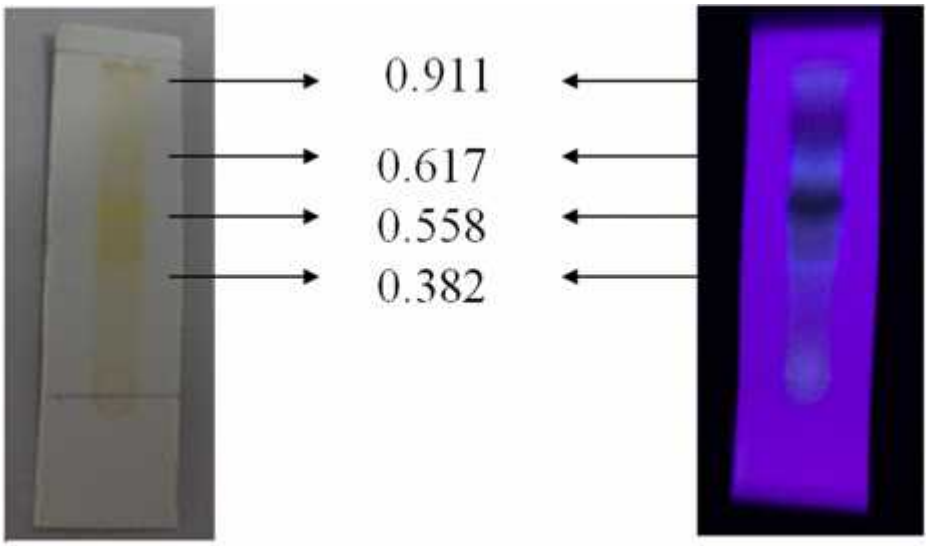


Fig. 6: Effect of methanol extract of Psoralea corylifolia on glycosylation of haemoglobin



UNDER IODINE *UNDER UV*

Fig. 7: Compound separation by thin layer chromatography

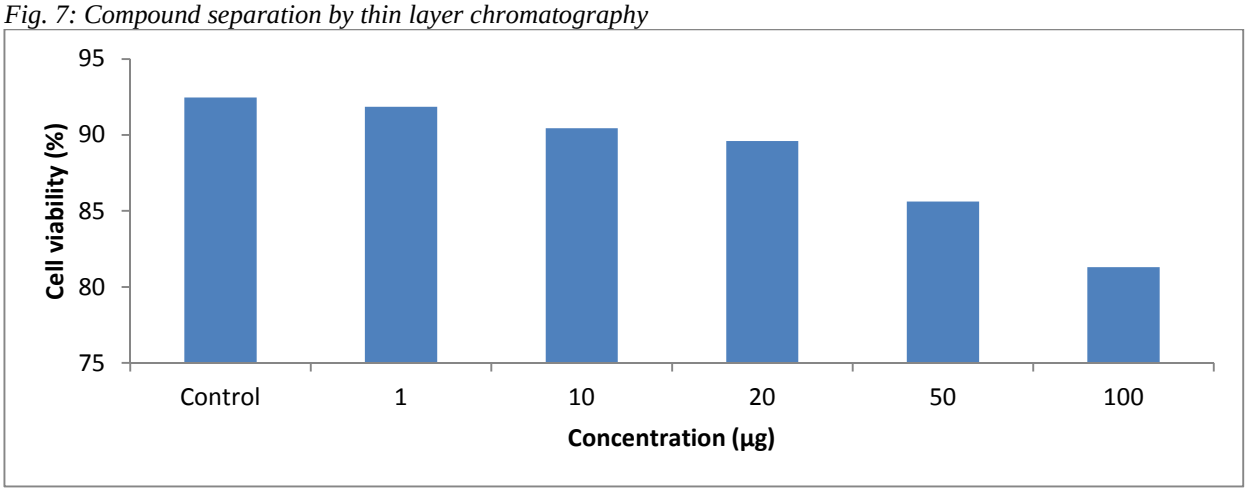


Fig. 8: Effect of methanol extract of P. corylifoila on L6 cell lines

Table 1: Qualitative phytochemical analysis

S.no	Compound	Result
1.	Alkaloids	+
2.	Phenols	+
3.	Glycosides	-
4.	Flavanoids	+
5.	Tannins	+
6.	Reducing sugars	+
7.	Saponins	-
8.	Proteins	-

cellulose) with 1ml of 0.15M sodium chloride containing 0.22M D – Glucose. The other end of the dialysis tube was sealed carefully. It was then placed in a beaker containing 45ml of 0.15M sodium chloride. The beaker was kept at room temperature and 1ml of external solution was collected after every 1hr [14]. The glucose concentration was estimated by DNS method of glucose estimation [13].

α- Amylase Inhibition Assay**:** To various concentrations of plant extract (250, 500, 750 and 1000µg/ml), 1ml 1% α-amylase solution was added and incubated for 30mins at 25°C. To 1ml of above mixture, 1ml of 1% starch solution was mixed and incubated for 3mins at 25°C. 1ml of DNS reagent was added to the mixture, diluted with 9ml distilled water and the absorbance was recorded at 540nm using a spectrophotometer [15]. The percentage inhibition of α amylase was calculated as,

The percentage inhibition of α amylase =

$$\frac{Abs_{sample} - Abs_{control}}{Abs_{sample}} * 100$$

$Abs_{control}$ = Absorbance of the control; Abs_{sample} = Absorbance of the sample

Glycosylation of Haemoglobin: Various concentrations of plant extract (250µg, 500µg, 750µg, 1000µg)was takesn in test tubes and 1ml each of glucose (2% in 0.01M Phosphate buffer) and haemoglobin (0.6% in 0.01M phosphate buffer) were added. The reaction mixture was incubated in dark condition at room temperature for 72 hrs. Absorbance was measured at 520nm [12]. The percentage increase in Glycosylation was given by the formula,

The percentage increase in Glycosylation =

$$\frac{Abs_{sample} - Abs_{control}}{Abs_{sample}} * 100$$

Qualitative Phytochemical Analysis: The phytochemical profile of the best screened extract was studied by using standard techniques proposed by Harborne (1984).

Quantitative Phytochemical Estimation: The total phenol content of the extract was measured at 765 nm by Folin

Table 2: Effect of methanol extract of *P. corylifoila* on L6 cell lines

S.No	Concentration (µg)	Cell viability (%)
1.	Control	92.47
2.	1	91.86
3.	10	90.45
4.	20	89.61
5.	50	85.63
6.	100	81.31

Ciocalteu reagent (McDonald *et al.,* 2001). Also, the total flavonoid content was studied by following the method prescribed by Yadav *et al.,* (2011).

Thin Layer Chromatography: The thin layer chromatography was done to determine the number of compounds present in the plant sample. The sample was placed in the pre coated TLC plate as a spot and was allowed to run with various solvent ratios. The solvents used were methanol and chloroform. Then the separation of compounds was viewed under a UV illuminator. R_f factor values were calculated.

$$R_f = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent front}}$$

MTT Assay for L6 Cell Lines: The MTT assay was performed based on the method given by Mosmann. 3-(4, 5 Dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) was used to test the viability of the cells. The cells were plated in each 100 µL of medium/well in 96-well plates. It was then allowed for incubation for 24 hrs in order to attach to the plate. The plant extract at different concentrations (1, 10, 20, 50, 100 µg) was added to the plates. By centrifugation, the cell medium was removed and replaced with 100 µl fresh media in each well containing the cells along with MTT. It was incubated for 4hrs. Supernatant was removed by centrifugation and 100 µl of isopropanol was added to all the wells. The absorbance was recorded using a microplate reader at 570nm. A well containing MTT without the plant extract was used as the control. The percentage of cell viability was calculated using the formula,

$$\text{Percent viability} = \frac{Abs_{sample} * 100}{Abs_{control}}$$

Abs_{sample} = Asorbance of the sample; $Abs_{control}$ = Absorbance of the control

RESULTS AND DISCUSSION

Glucose Uptake by Yeast Cells: This assay is based on the movement of glucose across the membrane of yeast cells, with the help of the plant extract. The yeast cells were suspended in plant extract and various concentrations of glucose (5mM, 10mM and 25mM). The plant extract enhances the yeast cells to take in the glucose. The amount of glucose remaining in the solution after incubation was observed. This determines the glucose uptake by the yeast cells. From the results, it was found that the percentage increase in glucose uptake by yeast cells at 5mM glucose concentration with methanolic extract ranges from 28 – 54% (Fig 1), ethyl acetate extract ranges from 13 – 37% (Fig 2), and hexane extract ranges from 12 – 26% (Fig 3). The percentage increase in glucose uptake at 10mM concentration with methanol extract ranges from 18 – 38% (Fig 1), ethyl acetate extract ranges from 12 – 32% (Fig 2), and hexane extract ranges from 6 – 23% (Fig 3). The percentage increase in glucose uptake at 25mM glucose concentration with methanol extract ranges from 1 – 23% (Fig 1), ethyl acetate extract ranges from 1 – 17% (Fig 2), and hexane extract ranges from 0.8 – 12% (Fig 3).

Glucose Diffusion Assay: The glucose diffusion test was performed by dialysis method. The concentration of glucose in the external buffer solution was measured. The plant extract enhances more amount of glucose to move through the dialysis membrane. Hence, the concentration of glucose in the external solution increases with time whereas the concentration of glucose inside the dialysis membrane decreased gradually. For every hour, 1ml of solution was collected and glucose estimation was done by DNS method. The concentration of glucose in the buffer ranges from 80 – 860 µg/ml with methanol extract of the plant material (Fig 4).

α- Amylase Inhibition Assay: α amylase is an enzyme that converts starch to glucose in its presence. When α amylase, glucose, plant extract are taken together as a solution, the plant extract causes the inhibition of enzyme activity. The percentage inhibition of α amylase increases from 52 to 78% with increasing concentration of plant extract (Fig 5).

Glycosylation of Haemoglobin: Haemoglobin forms a complex with glucose known as glycosylated haemoglobin. This reduces the concentration of free glucose in the blood. The plant extract plays a role in improving the formation of glycosylated haemoglobin. Hence for higher concentration of plant extract, less amount of free glucose remains in blood. This is estimated by calculating the % increase in Glycosylation of haemoglobin. The percentage increase in Glycosylation of methanol extract ranges from 10 to 94 ((fig 6).

Qualitative Phytochemical Screening: The presence of various compounds in the plant material was observed by certain phytochemical tests. The results of the qualitative phytochemical tests are presented in Table 1.

Quantitative Phytochemical Screening: Total phenol content and total flavanoid content were measured by a quantitative phytochemical test to be 1545 µgGAE/g sample and 1650 µgQE/g sample.

Thin Layer Chromatography: The TLC analysis of methanol extract of *P. corylifoila* revelead the presence of 5 compounds with R_f values 0.911, 0.617, 0.558 and 0.382 (Fig. 7).

MTT Assay for L6 Cell Lines: The methanol extract of *P. corylifoila* was found to cause a minimum toxicity on L6 cell lines. The effect was studied proportional to the extract concentration (Fig. 8). The cell viability was reduced to 81% by the extract (Table 2).

CONCLUSION

The incidence of diabetes is increasing day by day and it has become a major health concern today. Use of pharmacological agents for treating diabetes has proved to produce many side effects. Hence there is a need for an effective and a safe way for the treatment of diabetes. Medicinal plants are found to serve this purpose. The

antidiabetic efficacy of the plant *Psoralea corylifolia* was determined by using *in vitro* assays such as glucose uptake by yeast cells, glucose diffusion assay, α – amylase inhibition assay and glycosylation of hemoglobin assay. It was concluded that methanol extract of *Psoralea corylifolia* possess a higher antidiabetic activity. MTT assay for L6 cell lines was performed. This proved that the plant extract showed a minimum toxicity on the L6 cell lines. Furthermore, *in vivo* studies can be done to observe antidiabetic property of the plant much more effectively.

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