



***In Vitro* Antidiabetic Activity of Leaf Extracts of *Indianthus virgatus* (Roxb.) Suksathan and Borchs by Glucose Uptake Method**

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Received: 10 Mar 2019 / Accepted: 19 Apr 2019 / Published online: 1 Jul 2019

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Abstract

Medicinal plants play a key role to cure many diseases from time immemorial. The usage of medicinal plants in traditional medicinal system is the vital process of India. Diabetes Mellitus is a systemic metabolic disease characterized by hyperglycemia, abnormal elevated levels of lipid, and fat in blood and hypoinsulinaemia. The current epidemic of diabetes indicates the need of proper and effective medications that are limited in their potency to have many side effects. Thus, introduction of alternative and complementary medicine is now in picture.

Objective: The main objective of this work is to evaluate the *in vitro* anti diabetic activity of leaf extracts of *Indianthus virgatus* (Roxb.) Suksathan and Borchs in skeletal muscle cell line.

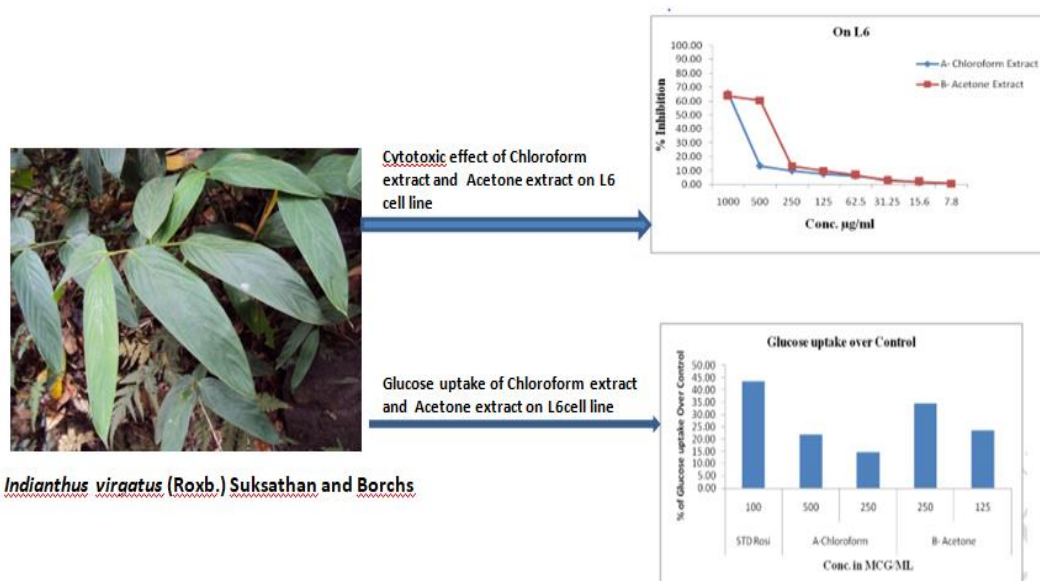
Methods: The *in vitro* cytotoxicity was performed for leaf extract (Chloroform and Acetone) on L-6 (Rat skeletal muscle) cell line to find toxic concentration of the leaf extract by MTT assay. Glucose uptake activity of test substance was determined in differentiated L-6 cells. **Result:** In Glucose uptake assay, acetone extract exhibited better activity with higher glucose uptake assay. Glucose uptake rate increased with the increasing concentration of the leaf extract.

Conclusion: The results of the current study clearly demonstrated the antidiabetic potency of leaf extracts obtained from *Indianthus virgatus* (Roxb.) Suksathan and Borchs under *in vitro* model.

Keywords

Indianthus virgatus (Roxb.) Suksathan and Borchs, Antidiabetic activity, *in vitro* cytotoxicity, MTT assay, Skeletal muscle Cell Lines.

PICTORIAL ABSTRACT



INTRODUCTION

Diabetes Mellitus is an established non communicable disease and often described as fourth or fifth leading cause of mortality in high income countries [1]. According to World Health Organization, 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. India presently has the largest number of diabetic patients in the world and has been infamously known as the diabetic capital of the world [2]. The classical symptoms of type 1 diabetes are polyuria (frequent urination), polydipsia (increased thirst), polyphagia (increased hunger), and weight loss. 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, headache, abnormal weight gain, allergic reaction, low blood glucose, dark urine, fluid retention, or swelling. Moreover, they are not safe for use during pregnancy [3]. Thus, the management of diabetes without any side effects is still a challenge. There is continuous search for alternative drugs [4]. As a result of the global epidemic of diabetes, the limited potency and many side effects of medications currently in use, the need for new diabetes therapies is expected to grow dramatically during the next decade. An intense research has been conducted to identify new therapeutic targets and pharmacologic compounds

that might correct the impaired glucose tolerance. During the recent years many investigators have shown that natural products are a potential source for new drug candidates for many diseases in general, and diabetes in particular. It is estimated that more than thousand plant species are being used as folk medicine for diabetes [5]. Herbal products or plant products are rich in flavonoids, phenolic compounds, coumarins, terpenoids and other constituents which help to reduce blood glucose levels [6]. Herbal drugs are prescribed due to their good effectiveness, fewer side effects in clinical experience and relatively low costs [7]. Active research has been performed on traditionally available medicinal plants for the discovery of new antidiabetic drug as an alternative [8].

Indianthus virgatus (Roxb.) Suksathan and Borchs. belonging to the family Marantaceae is locally known as 'Malamkoova'. *Schumannianthus virgatus* (Roxb.) Rolfe is a synonym of this plant. It is an erect herb of 4cm height with tuberous root stock. It has a compound leaf cluster on top of cane like stem. It is distributed in South India and Sri Lanka. In Kerala, the plants are abundant in Western Ghats. It is used by the Kurichar tribes to treat skin diseases [9]. It is also used by tribal healers of Kerala to treat jaundice [10]. The present study framed to investigate the *in-vitro* antidiabetic activity of leaf extracts of *Indianthus virgatus* (Roxb.) Suksathan and Borchs. by glucose uptake method.

MATERIALS AND METHODS

Collection and extraction of plant material

Fresh leaves of *Indianthus virgatus* (Roxb.) Suksathan and Borchs. was collected from Palode, Kerala, India and authenticated by Foundation for Revitalisation of Local Health Traditions herbarium, Bangalore, India. Leaves were cleaned and dried at room temperature for a period of 25 days under shade. Finely ground dried leaves were weighed and extracted using soxhlet apparatus by using acetone and chloroform as solvents.

Outline of the method

The *in vitro* cytotoxicity was performed for leaf extracts (acetone and chloroform) on L-6 (rat skeletal muscle) cell line to find toxic concentration of the extract by MTT assay.

Preparation of test solution

For cytotoxicity studies, 10mg of the test substance was dissolved separately and volume was made up with DMEM-HG supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by 0.22 μ syringe filtration. Serial two-fold dilutions were prepared from this for carrying out cytotoxic studies.

Cell line and culture medium

L-6 (rat skeletal muscle) cell line was procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in their respective media viz., DMEM-HG supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 g/ml) and amphotericin B (5 g/ml) in a humidified atmosphere of 5% CO₂ at 37 °C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm² culture flasks and all experiments were carried out in 96 well microtitre plates.

Cytotoxicity studies

The monolayer cell culture was trypsinized and the cell count was adjusted to 100,000 cells/ml using respective media viz., DMEM-HG containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, monolayer washed once with medium and 100 μ l of different test concentrations of test substances were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37°C for 72 h in 5% CO₂ atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval.

MTT ASSAY

After 72 h incubation, the drug solutions in the wells were discarded and 50 μ l of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37°C in 5% CO₂ atmosphere. The supernatant was removed and 100 μ l of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the standard formula and concentration of test substance needed to inhibit cell growth by 50% (CTC₅₀) values was generated from the dose-response curves for each cell line ^[11].

IN VITRO GLUCOSE UPTAKE ASSAY

Glucose uptake activity of test substance was determined in differentiated L-6 cells. In brief, the 24 hr cell cultures with 70-80% confluency in 40mm petriplates were allowed to differentiate by maintaining in DMEM with 2% FBS for 5-7 days. The extent of differentiation was established by observing multinucleation of cells. The differentiated cells were serum starved over night and at the time of experiment cells were washed with HEPES buffered Krebs Ringer Phosphate solution (KRP buffer) once and incubated with KRP buffer with 0.1% BSA for 30 min at 37°C. Cells were treated with different non-toxic concentrations of test and standard drugs for 30 min along with negative controls at 37°C. 20 μ l of D-glucose solution was added simultaneously to each well and incubated at 37°C for 30 min. After incubation, the uptake of the glucose was terminated by aspiration of solutions from wells and washing thrice with ice-cold KRP buffer solution. Cells were lysed with 0.1M NaOH solution and an aliquot of cell lysates were used to measure the cell-associated glucose. The glucose levels in cell lysates were measured using glucose assay kit (ERBA) ^[12]. Two independent experimental values in duplicates were taken to determine the percentage enhancement of glucose uptake over controls.

ABBREVIATION USED

FBS : Fetal bovine serum ; °C : Degree Centigrade ; % : Percentage ; gm : Gram ; hr : Hour ; mg : Milli gram ; mL : Millilitre ; nm : Nano meter ; μ l : Micro litre ; μ g : Micro gram ; MTT : 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; TPVG : Trypsin Phosphate Versene Glucose Solution; DMEM : Dulbecco's Minimum Essential Media; DMSO : Dimethyl sulfoxide; CTC₅₀ : Cytotoxicity concentration; EDTA : Ethylenediaminetetraacetic acid.

RESULTS AND DISCUSSION

Management of Diabetes Mellitus is a global problem. Successful treatment is very important for preventing or at least delaying the onset of long-term complications. Regulation of glucose level in the blood of the diabetic patient can prevent the various complications associated with the disease. The maintenance of plasma glucose concentration for a long term under a variety of dietary conditions is one of the most important and closely regulated processes observed in the mammalian species [13]. Through nature, drugs are available in the form of herbal medicines with very minimal adverse effects when compared to the available synthetic drugs to treat such chronic diseases and disorders. Such herbal drugs as therapeutic agents are a nature's boon when compared to the severe adverse effects of the allopathic medical practice for diabetes. Despite the fact that the search for a complete and

permanent cure for the disease is being pursued uncompromisingly by eluding physicians and researchers, many Indian Herbal Medicinal Plants have been noticed to be used successfully in managing Diabetes Mellitus and delaying or preventing its complications.

The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays. It depends both on the number of viable cells and on the mitochondrial activity of cells.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay is based on the assumption that dead cells or their products do not reduce tetrazolium. Tetrazolium salts are reduced only by metabolically active cells. Thus, MTT can be reduced to a blue coloured formazan by mitochondrial enzyme succinate dehydrogenase. The amount of formazan produced is directly proportional to the number of active cells [14].

[i]. Cytotoxic properties of test substance against L-6 cell line.

Sl. No	Name of Test Substance	Test Conc. (g/ml)	% Cytotoxicity	CTC ₅₀ (g/ml)
1.	Chloroform leaf extract	1000	65.82±0.42	849.34±3.29
		500	13.33±0.18	
		250	9.98±0.33	
		125	7.68±0.29	
		62.5	6.12±0.28	
		31.25	3.43±0.28	
		15.6	1.34±0.57	
2.	Acetone leaf extract	7.8	0.62±0.21	444.73±0.74
		1000	63.73±0.81	
		500	60.43±0.29	
		250	13.21±0.28	
		125	9.75±0.28	
		62.5	6.82±0.36	
		31.25	2.78±0.24	
		15.6	2.03±0.28	
		7.8	0.73±0.18	

GLUCOSE UPTAKE ASSAY RESULTS:

[ii]. In vitro glucose uptake studies for test substance in L-6 cell line

Sl no	Sample	Concentration (µg/ml)	Glucose uptake over control
1.	Chloroform leaf extract	500	21.75±2.18
		250	14.65±0.17
2.	Acetone leaf extract	250	34.46±0.28
		125	23.45±1.45

In the present study, acetone and chloroform extract of the leaf of *Indianthus virgatus* (Roxb.) Suksathan and Borchs were screened using MTT for its cytotoxicity against L6 cell line at different concentrations to determine the CTC₅₀ value [1].

The cytotoxicity of leaf extracts of *Indianthus virgatus* (Roxb.) Suksathan and Borchs were found to be dose dependent [1]. The acetone extract of this leaf did not confer any significant lethality to the healthy L6 cell line with a CTC₅₀ value greater than 444.73±0.74. The chloroform extract of this leaf did

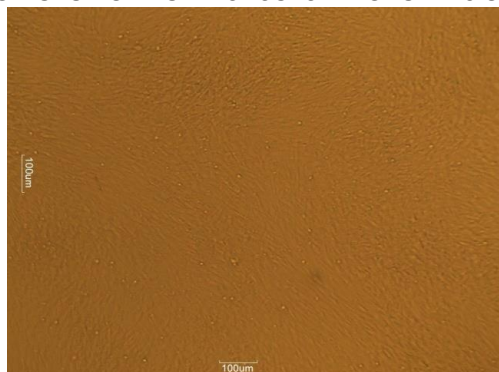
not confer any significant lethality to the healthy L6 cell line with a CTC_{50} value greater than 849.34 ± 3.29 . This confirms the safe nature of the extracts ^[ii].

Skeletal muscle is the primary responsible site for postprandial glucose use, and it is the most abundant tissue in the whole body. Defects in insulin-stimulated skeletal muscle glucose uptake are common pathological states in Type 2 diabetes (non insulin-dependent Diabetes Mellitus).

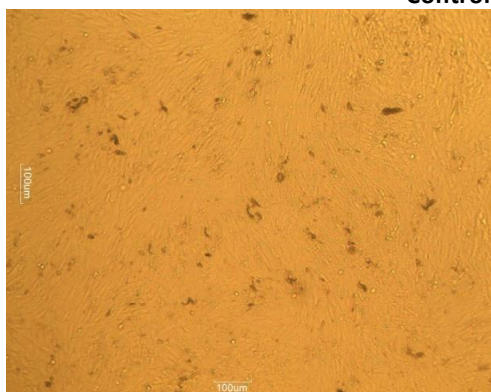
In the present study, acetone and chloroform leaf extracts of *Indianthus virgatus* (Roxb.) Suksathan and Borchs were evaluated for its *in vitro* anti diabetic

activity by glucose uptake method in skeletal muscle cell line. The test substance was evaluated for its *in vitro* anti diabetic activity by glucose uptake method in skeletal muscle cell line. In Glucose uptake assay, leaf extracts exhibited moderate toxicity to skeletal muscle cell line and glucose uptake assay it shows dose dependent glucose uptake over control ^[ii]. Glucose uptake rate increased with the increasing concentration of the leaf extract. Chloroform extract exhibited moderate glucose uptake whereas acetone extract exhibited better activity with higher glucose uptake ^[iii].

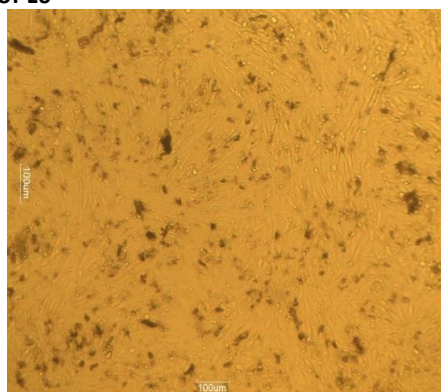
I. CYTOTOXICITY OF TEST SUBSTANCE ON L-6 CELL LINE.



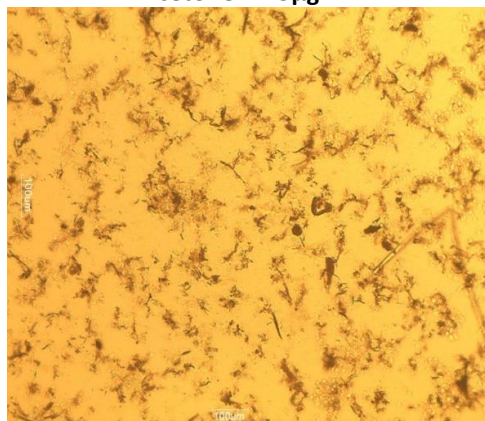
Control of L6



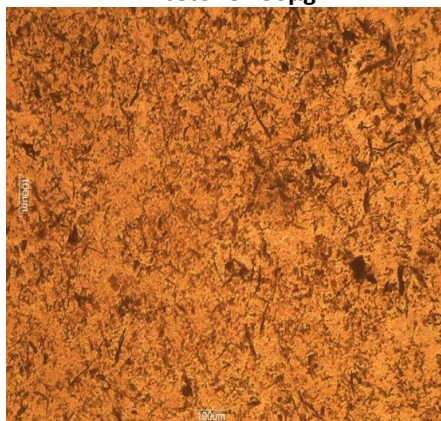
Acetone -125µg



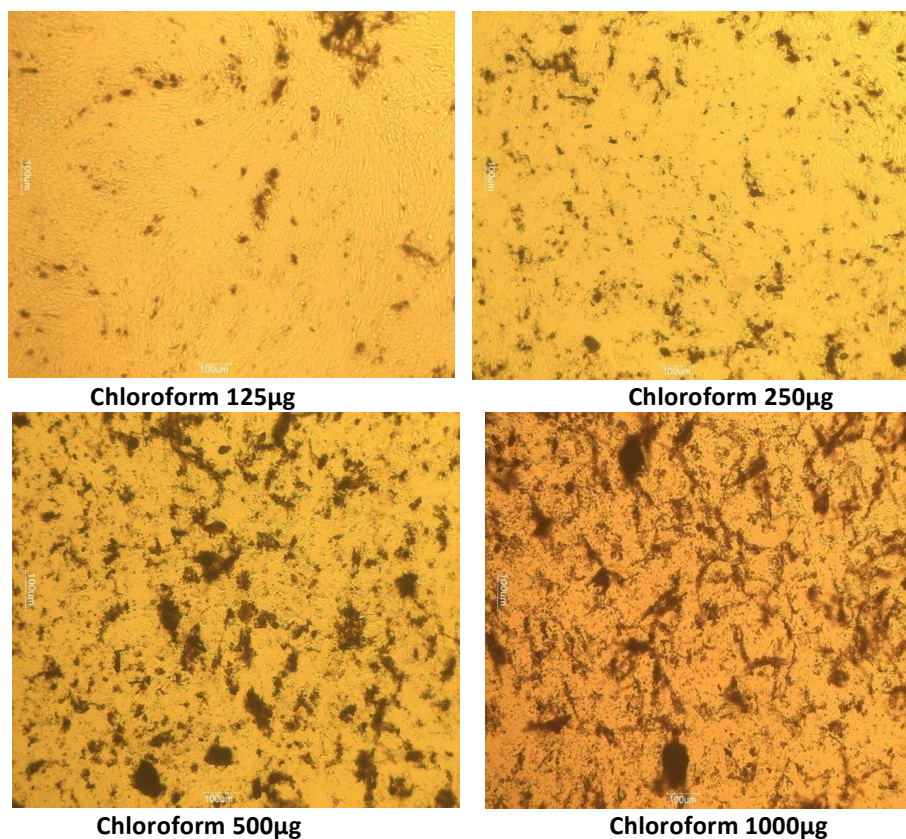
Acetone 250µg



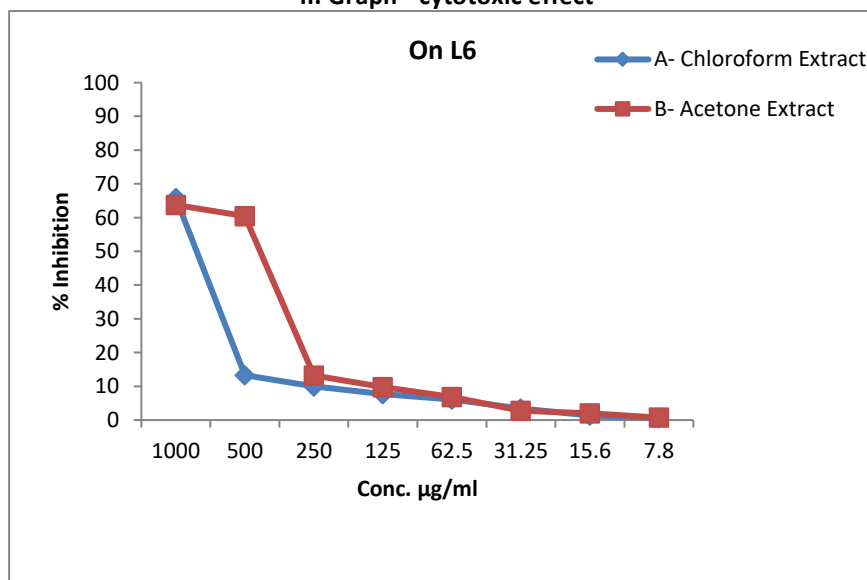
Acetone 500µg



Acetone 1000µg

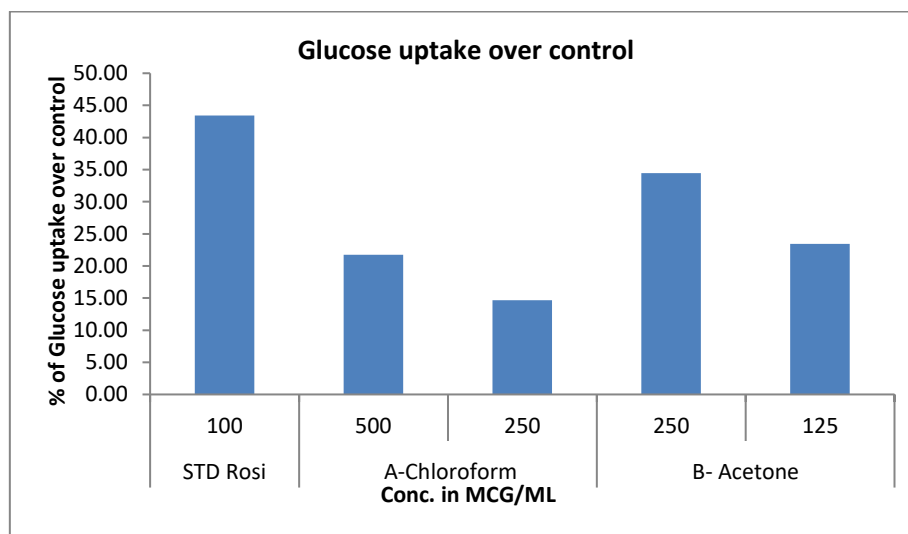


II. Graph - cytotoxic effect



Graph of cytotoxic effect of A-Chloroform Extract and B- Acetone Extract on L6 cell line

II. Graph - Glucose uptake



Graph of Glucose uptake of A-Chloroform Extract and B-Acetone Extract on L6 cell line

SUMMARY

Skeletal muscle is a major tissue involved in glucose uptake. The L-6 cell line is the best characterized cellular model for glucose uptake because they have been used extensively to elucidate the mechanism of glucose uptake in muscle. The test substance according to CTC₅₀ was evaluated for their *in vitro* anti diabetic activity in skeletal muscle cell line. A dose dependent stimulated glucose uptake by test substance to the cell surface was estimated; where test substances, chloroform leaf extract exhibited moderate glucose uptake whereas acetone extract exhibited better activity with higher glucose uptake enhancement of 34.46µg/ml and 23.45µg/ml at 250 and 125 µg /ml, respectively over control.

CONCLUSION

The results of the current study clearly demonstrated the antidiabetic potency of acetone and chloroform leaf extracts obtained from *Indianthus virgatus* (Roxb.) Suksathan and Borchs. under *in vitro* model. Acetone extract exhibited better glucose activity with higher glucose uptake compared to chloroform. We can therefore conclude from this study that this plant may essentially contain herbal bioactive compounds which require further structural elucidation and characterization methodologies to identify the bioactive constituents. However, *in vivo* studies have to be carried out to substantiate the *in vitro* results by employing different *in vivo* models and clinical trials for their effective utilization as therapeutic agents.

ACKNOWLEDGEMENT

The authors are grateful to Bharathiar University, Research and Development centre, Coimbatore, Tamil Nadu and St. Joseph's Postgraduate and Research Centre, Langford Road, Bengaluru, Karnataka, India and for providing necessary support, guidance and facilities to do the research.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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