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EVALUATION OF *IN-VITRO* ANTIDIABETIC ACTIVITY OF *CARALLUMA ATTENUATA WIGHT*

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ABSTRACT

Caralluma attenuata Wight belonging to family Asclepidaceae is a succulent herb. In the present study *In-vitro* anti diabetic properties were determined. Determination of glucose adsorption capacity, Effect of Caralluma attenuata extract on *In-vitro* glucose diffusion and Glucose uptake by yeast cells were used to determine the in-vitro anti diabetic properties of *Caralluma attenuata*. The results reveal that the alcoholic extract of Caralluma attenuata possess a significant anti diabetic properties *In-vitro* models it is observed that increase in concentration of alcoholic extract of *Caralluma attenuata* are able to produce increase in anti-diabetic properties.

KEY WORDS: Diabetes, *Caralluma attenuata*, anti-diabetic activity, *In-Vitro,* Glucose Adsorption Capacity, Glucose Diffusion.

1. INTRODUCTION

1.1 Diabetes

According to the American diabetes association "Diabetes is a group of metabolic disease characterised by hyperglycemia resulting from the defects in the Insulin secretion and action or both. Diabetes mellitus was identified and measured by the symptoms of polyuria and weight loss. The Greek physician "***Aertaeus"*** has given term diabetes mellitus (DM). Diabetes mellitus is called as "***Madhumeha"*** by the ancient Hindu physicians. Madhumeha is a disease condition in which the patient produces sugariness from the whole body, i.e., in the form of sweat, blood and breath etc., and the patient passes the urine which contains high level of glucose. (Neeta *et al*., 2017). The symptoms of diabetes mellitus are polydipsea, polyuria, poly phagia, fatigue, nausea, vomiting, impotence in men, slow healing wounds and blurred vision. (Verma *et al*., 2018)

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Since insulin is been produced by the β cells of the islets of Langerhans, any receding in the number of functioning cells will decrease in the amount of insulin that can be synthesised. Many diabetics can produce sufficient amount of insulin but some stimulus to the islets tissue is needed in an order so that secretion can take place. During the early stages of this disease the Insulin like Activity (ILA) of the blood is often increased, but most of this insulin appears to be bound in protein and is not available for the transport across cell membrane and for the action of the cell. (Srilakshmi, 2011)

The clinical diabetic status is defined by the perpetuated elevation of blood glucose cluster. The cluster of glucose in diabetes is often significantly overshoots the higher to the standard limit or the level. The most recommended methods to detect the diabetic mellitus are determined by the level of glucose in blood at the factors given below.

- Fasting plasma glucose 126mg/dL
- Random plasma glucose 200mg/dL
- Oral glucose tolerance test >200mg/dL (Neeta *et al*., 2017)

Taxonomical classification

Kingdom: Plantae
Sub kingdom: Tracheobionta
Division: Magnoliophyta
Class: Magnoliopsida
Sub Class: Asteridae
Order: Getianaies
Family: Asclepidaceae
Genus: *Caralluma*
Species: *attenuate*

Vernacular Names

Tamil: *Kallimulaiyan*
Telugu: *kundellu kommulu, kundeti kommulu*
Sanskrit: *Yugmaphalothama*

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Caralluma attenuata (Asclepiadaceae) is a very variable herb up to 1m in height, perennial and succulent herb which is widely distributed in India from Andhra Pradesh and Maharashtra to Kerala up to 600m and other countries. (Anonymous, 1992; 1999) It is a thick fleshy herb which is edible with almost leafless stem, the corolla banded with white or green and purple on the tube and on the lower part of the lobes, the upper part is dark purple and with purple fringes (Anonymous, 1992;Gamble, 1957; kirtikar *et al.,* 1996).

Vijay Jyothi *et al*., 2018 has reported that methanolic stem extract of *Caralluma adscendens var Caralluma attenuata* has significant antioxidant activity and hepatoprotective.

Chandra Mohan *et al*., 2017 has reported that flavonoids fraction of *C.attenuata* was tested for their potential **anti-diabetic activity** by conducting *invitro* test and the results of invitro α-amalylase and α-glucosidase inhibition test had stated that *C.attenuata* flavonoids fraction had a similar inhibitory effect with that of standard drug acarbose.

Muthulingam, 2017 has reported that aqueous extract of *C.attenuata* was investigated for its **antioxidant and lipid peroxidative efficacy** on antituberculosis drug rifampicin.

Muthulingam, 2017 has reported that *C.attenuata* has a significant traditional property in treating liver disorder by evaluating rifampicin induced hepatotoxicity in rats.

Vijaya Jyothi *et al.,* 2017 has reported that the methanolic stem extract of *C.attenuata* aqueous methanol (50:50) has ***invitro*** **antioxidant property** which was confirmed by performing free radical scavenging assay, reductive ability and nitric oxide method.

Vijaya Jyothi *et al*., 2016 has reported that *C.attenuata* of aqueous methanolic extract (50:50) has several biological activities like **anti- tubercular** by micro plate alamar blue assay method against ciprofloxacin, pyrazinamide which showed significant activity at 50µg/ml. whereas **anthelminthic activity** was evaluated on *perithima posthuma (*earthworms) using piperazine citrate as standard drug which showed significant activity at 300 mg/ml.

Garg *et al*., 2016 has reported that *Caralluma attenuata* can be used as **antiulcer agents.**

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Garg *et al*., June 2014 has reported that the *C.attenuata* can be used in treating **ulcer** by conducting different ulcer models studies on rats.

Pradeep Kumar *et al*., 2013 has reported that the *C.attenuata* ethanol has a potent **anti-diabetogenic effect** and **antioxidant effect** in diabetic rats significant activity

Kalaivani *et al*., 2011 has reported that the *C.attenuata* extract have a **antihyperglycemic effect.**

Jayakar *et al*., 2004 they have reported that **hypoglycemic effect** of aqueous and alcoholic extract of whole plant *C.attenuata.*

Venkatesh *et al.,* April 2003 has reported that *C.attenuata* has been screened for their **antihyperglycemic activity.**

2. Materials and methods:

2.1 Collection and identification

The whole plant *Caralluma attenuata Wight* was collected on 29th of October 2019 from the rocky areas of Ghatkesar, Hyderabad.

The plant material was identified and authenticated by comparing with available literatures: - Compendium of Indian Medicinal Plant [volume3], Reviews on Indian Medicinal Plant [volume 5], Wealth of India [volume 3], Flora of the Presidency of Madras [volume 2] and further plant was compared for its identity with voucher specimen (GPR-CA-99) which is being maintained in the laboratory of Pharmacognosy and Phytochemistry at G. Pulla Reddy College of Pharmacy, Mehdipatnam, Hyderabad.

The collected whole plant of *Caralluma attenuata Wight (*1.56kgs) were washed thoroughly to remove foreign matters like small stones, dust particles and mud etc., The stem part were chopped into small pieces and grinded into a smooth paste using electric blender.

2.1.1 Extraction:

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The smooth paste of plant material (1.479kgs) was macerated with absolute alcohol. After 10days of maceration the solvent was filtered and alcohol is recovered under reduced pressure using Rotary Flash evaporator. The dried concentrated extract was stored in desiccator.

2.2 EVALUATION OF *IN-VITRO ANTI*-DIABETIC ACTIVITY

Evaluation of antidiabetic activity of *Caralluma attenuata alcoholic* extracts was carried out using various *in-vitro* methods. Following are the some of the *in-vitro* methods used in the present study to determine the antidiabetic activity of *Caralluma attenuata* extract.

- Determination of glucose adsorption capacity
- Effect of plant extract on *in-vitro* glucose diffusion
- Glucose uptake by yeast cells

2.2.1 Determination of Glucose Adsorption Capacity:-

Requirements: GOD-POD glucose diagnosing kit, glucose, shaker water bath, auto analyser

Preparation of stock solution Glucose Solution

About 18.016gm of glucose was weighed and dissolved in small amount of distilled water then diluted up to 100ml to make 1M/1000mM (solution stock solution).

Preparation of series of dilutions

From the stock solution dilutions were made to prepare 5mM (0.005M), 1OmM (0.01M), 20mM (0.02M), 50mM (0.05M) and 100mM (0.1M) solution i.e., 0.5ml, 1ml, 2ml, 5ml, and 10ml of the stock solution was taken and diluted up to 100ml with distilled water to get 5, 10, 20, 50 and 100mM glucose solution respectively.

From each above series of dilutions 10µl of the solution was taken into the ephendroff's tubes separately to which 1ml of working reagent was added (prepared from GOD-POD kit) and observed for original glucose concentration i.e., G_1 using auto analyser.

Preparation of 1% Extract solution

250mg of the extract was weighed and dissolved small amount of distilled water and sonicated, when necessary, then diluted to 25ml to make it 1% of the solution.

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Procedure

To 25ml of the each 5, 10, 20, 50 and 100mM of glucose solution required amount of extract was added to make it about 1% of the solution (250mg extract for 25ml of the solution) which was mixed properly. The mixture was then incubated on a shaker water bath which was maintained at 37°c for 6hrs for thorough mixing of the solution.

After 6hrs of the incubation the mixture was collected separately in ephendroff's tubes and centrifuged at 4000rpm for 20minutes. The supernatant was analysed using GOD-POD kit in an auto analyser for the determination of glucose bond using the given formula. (Ou *et al*., 2001; Bhutkar *et al.,* 2018)

Formula:

The amount of glucose bound was determined by using given formula and the results were expressed in the terms of mM/L.

$$\textbf{Glucose bound} = \frac{G1-G6}{\text{weight of the sample}} \text{ X volume of solution}$$

G_1 = original glucose concentration

G6 = Glucose concentration after 6 hours

2.2.2 Glucose uptake by yeast cells

Requirements: GOD-POD kit, Glucose, Bayer's yeast

Preparation of 10% v/v yeast suspension

Bayer's yeast was washed repeatedly by centrifugation in distilled water until the supernatant fluids were clear. The volume of yeast was determined after centrifugation at 3000 x g for 5min. The suspension was prepared by taking 10ml of washed yeast and diluted it to 100ml with distilled water (Cirillo, 1962)

Preparation of 10mM glucose solution

180mg of the glucose was weighed and dissolved in small amount of distilled water then diluted up to 100ml.

Preparation of Extract solution

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250mg of the extract was weighed and dissolved in small amount of distilled water, sonicated when necessary and diluted up to 50ml to get 5mg/ml (5000µg/ml) concentration solution. Further series of dilutions were made by withdrawing 2ml, 4ml, 6ml and 8ml from 5mg/ml concentration of solution and diluted up to 10ml with distilled water to get 1, 2, 3 and 4mg/ml concentration respectively.

Procedure

1ml of each concentration of the extracts (1 to 5mg/ml) was mixed with 1ml of glucose solution and incubated at 37°c for 10min. After 10min of incubation 100µg/ml of yeast suspension was added and again incubated for 1hour at 37°c temperature. After 1 hour of incubation the solutions were subjected to centrifugation for 5minutes at 2500xg rpm. Supernatant was collected for glucose content determination using GOD-POD kit. Simultaneously a control was also performed without adding sample. (Cirillo, 1962; Nair *et al*., 2013)

Formula:

Increase in the percentage of glucose uptake by yeast cells was calculated by the given formula:

$$\text{Increase in Glucose Uptake (\%)} = \frac{Absorbance\ control - Absorbance\ sample}{Absorbance\ control} \times 100$$

2.2.3 Effect of *Caralluma attenuata* on *In-Vitro* Glucose Diffusion

Requirements: GOD-POD glucose kit, glucose, Franz diffusion cell, dialysis membrane.

Preparation of 20mM (0.02M) glucose solution

18.016 g of glucose was weighed dissolved in small amount of distilled water and diluted up to 1M concentration. 2ml of 1M concentration was diluted up to 100ml with distilled water to make 20mM solution.

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Preparation of 1% Extract solution

250mg of the extract was weighed and dissolved in small amount of distilled water and diluted up to 25ml to make 1% concentration and sonicated when necessary.

Procedure

The acceptor compartment of Franz diffusion cell was filled with water up to the brim. The donor compartment was filled 25ml of the glucose solution (20mM) was mixed with required quantity of the extract such that it constituents 1% concentration up to the mark. A dialysis membrane was placed between the donor and acceptor compartments of the diffusion cell. The whole assembly was placed on a magnetic stirrer maintained at 37°c for 3hours. At different time intervals like 30, 60, 120 and 180 min samples were withdrawn through the sampling port which is attached to the acceptor compartment and glucose content was determined by using GOD-POD kit. A control was also performed simultaneously. (Ou *et al.,* 2001)

Formula:

The Glucose dialysis retardation index was calculated using the given formula

$$\mathrm{GDRI} = 100 - \frac{\text{Glucose content with addition of sample } \left(\frac{mg}{dl}\right)}{\text{Glucose content of the control } \left(\frac{mg}{dl}\right)} \times 100$$

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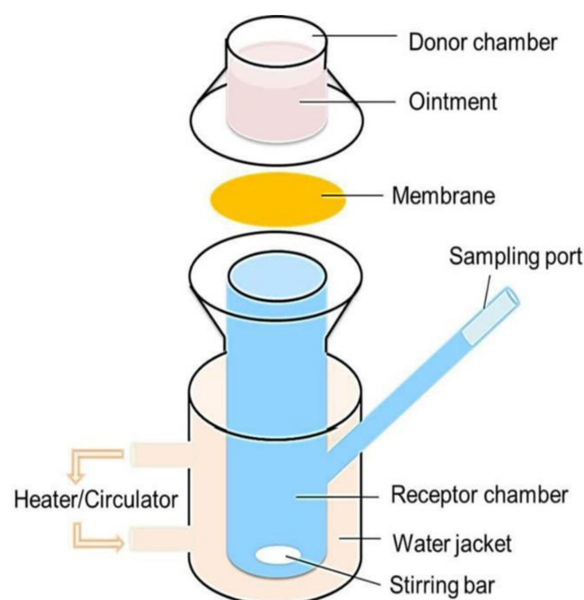

Figure: 1 Franz's cell diffusion

3. Results and Discussion:

3.1 Extraction: Fresh whole plants of *Caralluma attenuata Wight* were collected from the rocky areas of Ghatkesar, Hyderabad in the month of October 2019 from Ghatkesar, Hyderabad. The stems were chopped into small pieces and grinded into a smooth paste using electric blender (1479grams) and extracted with absolute alcohol to yield 33grams (2.23%).

3.2 Results of *In-Vitro* Anti-Diabetic Activity

Various methods are available for evaluating anti-diabetic activity of a sample *in-vitro.* Some of them are glucose adsorption capacity, *in-vitro* glucose diffusion and glucose uptake by yeast cells methods were used to evaluate the *in-vitro* anti-diabetic property of the *Caralluma attenuata Wight* plant.

3.2.1 Glucose Adsorption capacity of *Caralluma attenuata* extract

The extract of *Caralluma attenuata* was incubated with glucose solution of different concentration for 6 hours and analysed for glucose concentration. The difference in the glucose content before and after incubating with extracts represents the adsorption capacity. The glucose binding capacity was observed to be increasing with increasing concentration.

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The glucose with 100mM concentration is 6.879±0.0005044. Figure 2 & Table 1,2 shows the results of glucose adsorption capacity of alcoholic *Caralluma attenuata* extract. The adsorption capacity can be attributed to presence of fibers in the plant as fibers are reported to adsorb glucose (Ou *et al.,* 2001; Chau *et al.,* 2004).

If a test sample can adsorb glucose, it reduces the amount of glucose available for transport across the intestinal lumen ultimately lowering postprandial hyperglycemia. Higher the glucose adsorption capacity more will be the hypoglycemic potential.

Table 1 Glucose concentration before and after incubation with *Caralluma attenuata*

Extract	Glucose conc. Used	G_1 (mg/dl)	$G_{6(}$mg/dl)
Alcoholic extract	5mM	80.23	51.98±0.008819
	10mM	156.5	72.89±0.008819
	20mM	234.1	137.8±0.05774
	50mM	775.8	193.5±0.1453
	100mM	1427.0	187.8±0.08819

The values are mean ± SEM of triplicate determination.

$G_{1:}$ original glucose concentration; $G_{6:}$ glucose concentration after 6hrs of incubation.

Table 2 Glucose Adsorption by *Caralluma attenuata*

Extract	Conc. Glucose solution	Glucose bound (mg)	Glucose bound (mM/L)
Alcoholic extract	5mM	2.85±0.0008819	0.1568±0.0005
	10mM	8.361±0.0008819	0.4641±0.0005

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	20mM	9.630±0.005774	0.5345±0.0003180
	50mM	58.23±0.01453	3.231±0.0007024
	100mM	123.9±0.008819	6.879±0.0005044

The values are mean ± SEM of triplicate determination

Glucose concentration in mM = Glucose concentration in mg X 0.05551

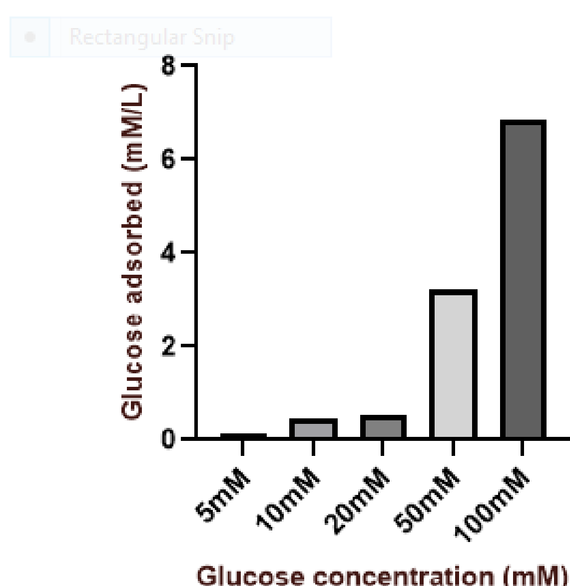

Figure 2: Graph of glucose adsorption capacity of *Caralluma attenuata* at different concentrations

3.2.2 Glucose uptake by Yeast cells

Caralluma attenuata extract was incubated with glucose solution and 10% yeast suspension along with negative control for 1 hour, centrifuged, analysed for glucose content and compared with control. Effect of *Caralluma attenuata extract* on glucose uptake up yeast cells is shown in figure-3 and table-3, 4. The *Caralluma attenuata* showed different degrees

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of glucose uptake across yeast cells. The maximum uptake was 31.64% at 5mg/ml concentration.

The studies on transport of non-metabolizable sugars and certain metabolizable glycosides have suggested that sugar transport across yeast cells is mediated by stereospecific membrane carriers. It is also reported that in yeast cells, glucose transport is extremely complex and glucose is transported by a facilitated diffusion process using facilitated carriers which transport the solutes down the concentration gradient, implying the effective transport is only attainted if there is removal or utilisation of intracellular glucose (Cirillo *et al.,* 1962; Bhutkar *et al.,* 2016).

Table 3 Effect of *Caralluma attenuata* extract on glucose uptake by yeast cells

Sample	**1mg/ml**	**2mg/ml**	**3mg/ml**	**4mg/ml**	**5mg/ml**
Alcoholic Extract	0.678	o.577	0.517	0.530	0.512

Absorbance of control: 0.749

Values are mean of triplicate determination.

Table 4 Percentage increase in glucose uptake in presence of *Caralluma attenuata* extract

Sample	**1mg/ml**	**2mg/ml**	**3mg/ml**	**4mg/ml**	**5mg/ml**
Alcoholic Extract	9.47%	22.96%	30.97%	29.23%	31.64%

Values are means of triplicate determination.

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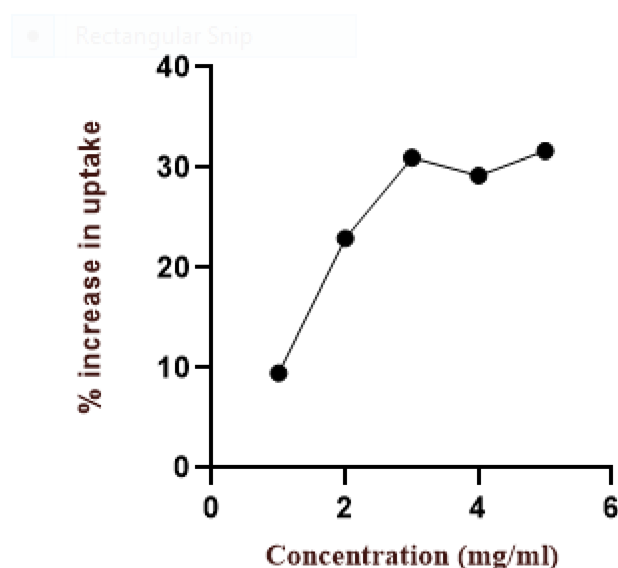

Figure 3 Graph of effect of *Caralluma attenuata* on uptake of glucose by yeast cells

3.2.3 Effect of *Caralluma attenuata* on *In-Vitro* Glucose Diffusion

10Mm glucose solution was allowed for diffusion across the dialysis membrane with extract simultaneously with control (without extract), for 3hours and glucose content in dialysate was analysed at different intervals of 30, 60, 120 and 180 min. Effect of *Caralluma attenuata* extract on retardation of glucose diffusion across dialysis membrane is shown in tables 5 and 6.

GDRI is a useful *in-vitro* index to predict the effect of fiber on delay in the glucose adsorption in the GI tract. In the present investigation, the GDRI value differs significantly for the extract at different time intervals and was observed to decrease over a period of time. The alcoholic extract of *Caralluma attenuata* showed higher significant ($p \leq 0.05$) GDRI at 60min, whereas after 60mins of time interval it started showing diminishing or decreased in the significant values of GDRI as shown in the figure-4.

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Table 5: Effect of *Caralluma attenuata* extract on *In-Vitro* Glucose Diffusion

Sample	Glucose content in dialysate (mM)			
	30min	**60min**	**120min**	**180min**
Control	0.6277± 0.0003333	0.4513± 0.0003333	0.4587± 0.0003333	1.363±0.0003333
Alcoholic extract	2.432± 0.002848	1.927± 0.0003333	1.639± 0.0003333	1.114± 0.0003333

Values are means ± SEM of triplicate determination ($p \leq 0.05$)

Glucose concentration in mM = Glucose concentration in mg X 0.05551

Table 6: Glucose dialysis index of *Caralluma attenuata* extract in glucose diffusion

Sample	GDRI			
	30min	**60min**	**120min**	**180min**
Alcoholic Extract	287.4± 0.1453	326.5± 0.2309	257.3± 0.2028	-18.31± 0.005774

Values are means ± SEM of triplicate determination ($p \leq 0.05$)

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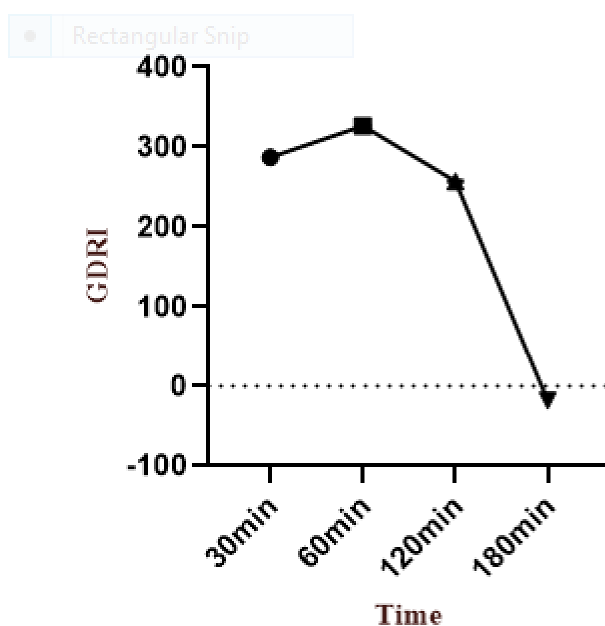

Figure 4: Graph of GDRI of *Caralluma attenuata* at different time intervals.

CONCULSION

Caralluma attenuata showed maximum *in-vitro* antidiabetic activity in all the three methods at different concentration. The adsorption of glucose may be due to the presence of fibers as they are reported for their glucose binding capacity (Ou *et al.,* 2001; Chau *et al.,* 2004). Binding reduces the amount of glucose available for transport across the intestinal membrane; causing reduction in postprandial hyperglycemia. In glucose adsorption capacity method, glucose binding capacity was observed to be increasing with increasing concentration. The glucose with 100mM concentration is 6.879±0.0005044. The mechanism of glucose transport across the yeast cell membrane has been receiving attention as an important *in-vitro* screening method for hypoglycemic effect of various test substances. The amount of glucose remaining in the external medium after specified period of incubation is an indicator of amount of glucose uptake by yeast cells. The *Caralluma attenuata* extract showed increase in glucose uptake by yeast cells, maximum uptake value 31.64% at 5mg/ml concentration.

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Single glucose transport in yeast cells is a facilitated process; increase in uptake indicates utilization of intracellular glucose by the cells.

Inhibitory effect against in-*vitro* glucose diffusion through dialysis membrane was also observed. GDRI value decreases overtime, *Caralluma attenuata* it showed higher significant ($p \leq 0.05$) value in the order of 60min, 30min and also at 120min. And further it showed decrease in the activity. All three *in-vitro* screening method for antidiabetic activity used in the present investigation are having different kind of mechanism, which help in understanding the mechanism of action of *Caralluma attenuata Wight* plant in the treatment of diabetes. Thus the plant *Caralluma attenuata Wight* was verified to possess significant antidiabetic action *in-vitro.* This action may be due the presence of various phytoconstituents present including fibers. The suggested mechanism of action can be by adsorption, inhibition of diffusion of glucose and majorly by increasing utilization of intracellular glucose. Further there is a scope of this work which can be achieved by performing other *in-vitro* test like alpha-glucosidase inhibition assay and alpha-amylase inhibition assay studies to develop a potential candidate in the treatment of diabetes.

LIST OF ABBREVIATIONS

gram (G), centimeter (cm), micro gram (µg), milli gram (mg), milli moles (mM), Deci liter (dl), Liters (L), Percentage (%), Degree Celsius (°c), World health organization (WHO), Minute (Min), Rotation per minute (Rpm), Glucose oxidase -peroxidase kit (GOD-POD kit).

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