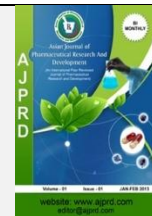


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Research Article

### Phytochemical Investigation and In Vitro Antidiabetic Activity of *Melothria Scabra*

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#### ABSTRACT

The family Cucurbitaceae includes a large group of crops like cucumbers, and melons which are medicinally essential. The major elements present are the phytochemicals like Terpenoids, Saponins, Tannins, Steroids, Carotenoids, Glycosides and Resins etc and the most commonly the terpenoid substance called cucurbitans. *Melothria scabra* belongs the family Cucurbitaceae. The leaves of *Melothria scabra* are dried and powdered. Extraction was performed by using ethanol by soxhlation method. In the present study, the phytochemical investigation and invitro anti diabetic activity was performed. It indicates the presence of Carbohydrates, Proteins, Alkaloids, Flavanoids and Glycosides. Powder analysis is performed by using dried powder. It indicates the presence of Stomata, Xylem vessels and Fibres. These extracts were screened for invitro anti diabetic activity by using the three methods: Determination of glucose adsorption capacity, Effect of plant extracts on invitro glucose diffusion, Glucose uptake by yeast cells. The plant extract has shown good increase in glucose adsorbing capacity, decrease in glucose diffusion retardation potential and significant decrease in glucose uptake thus indicating its ability to decrease the glucose availability to diffusion into blood stream.

**Keywords:** Diabetes Mellitus, Glucose Diffusion, In vitro Antidiabetic activity, Cucurbitaceae

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#### INTRODUCTION

Diabetes mellitus is a disease in which the body is ability to produce or respond to the hormone insulin is impaired resulting in abnormal metabolism of carbohydrates and elevated levels of glucose in blood<sup>[1]</sup> 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<sup>[2]</sup>. India presently has the largest number of diabetic patients in the world and has been infamously known as the diabetic capital of the world<sup>[3]</sup>. 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,  $\alpha$ -glucosidase inhibitors and 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<sup>[4]</sup>. Active research has been performed on traditional available medicinal plants for discovery of new antidiabetic drug as an alternative for synthetic drugs. Hence the current study is focused to evaluate the antidiabetic potential of selected medicinal plants.

After the thorough literature review we have found that the plants in Cucurbitaceae Family have tremendous medicinal

properties such as anti-HIV, anxiolytic, anti-pyretic, anti-diarrhoeal, carminative, antioxidant, anti-diabetic, antibacterial, laxative, anthelmintic, anti-tubercular, purgative and hepatoprotective. It is also employed as an abortifacient, diuretic, and cardiotonic agent. They also show strong anti-inflammatory, antitussive, cytotoxic, and expectorant properties. Apart from biological profile Cucurbitaceae family posses many therapeutically important chemical constituents which required further research to explore the medicinal value of this species<sup>[5]</sup>. Hence the objective of the study is to Investigate the phytochemical Content and Invitro evaluation of antidiabetic activity of *Melothria Scabra*.

### Materials and Methods:

The study was conducted as a part of Academic research in Vaageswari College of Pharmacy in December 2017

**Collection of plant materials:** The leaves of *Melothria scabra* was collected from the local areas of Karimnagar, Telangana, India.

**Preparation of the extracts:** The collected leaves of *Melothria scabra* were washed and air dried in the shade at room temperature for complete drying. The dried sample was powdered 10g of powder was packed in a thimble, and it was extracted into ethanol using a Soxhlet apparatus. After extraction, the solvents were evaporated to dryness and the yields of the extracts were calculated. They were stored at -20°C until use. Apart from the solvent fresh aqueous extract was also prepared.

### METHODS

#### Determination of glucose adsorption capacity:

Glucose adsorption capacity of the samples was determined by the method of Ou et al. Briefly, the samples of plant extracts(1%) were added to 25ml of glucose solution of increasing concentration(5,10,20,50, and 100mmol/L). The mixture was stirred well, incubated in a shaker water bath at 37°C for 6hrs, centrifuged at 4800r/minutes for 20 minutes and the glucose content in the supernatant was determined.

### RESULTS

**Table1:** Pharmacognostic Studies: Phytochemical Screening

Constituents	Method	Result
Carbohydrates -Molish test	2-3ml aqueous extract + few drops of $\alpha$ -naphthol+alcohol+conc.H <sub>2</sub> SO <sub>4</sub>	+ve
Proteins -Biuret test	3ml of test solution+4%NaOH+few drops of 1%CuSO <sub>4</sub>	+ve
Alkaloids -Mayer's test	2-3ml of filtrate + few drops of mayer's reagent	+ve
Flavanoids	Residue + lead acetate	+ve
Glycosides - Salkowski test	2ml extract+2ml CHCl <sub>3</sub> +2ml conc.H <sub>2</sub> SO <sub>4</sub> +shake well	+ve

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.

Gliclazide was used as a Standard. The concentration of bound glucose was calculated using the following formula:

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

G1 is the glucose concentration of the original solution.

G6 is the glucose concentration after 6h.

#### Effect of plant extracts on in vitro glucose diffusion:

It was performed according to the method stated by Ahmed et al. A total of 25ml of glucose solution (20mmol/L) and the samples of plant extracts (1%) were dialyzed in dialysis bags against 200mL of distilled water at 37°C in a shaker water bath. The glucose content in the dialysate was determined at 30, 60,120 and 180 minutes using glucose oxidase peroxidase diagnostic kit. Gliclazide was used as a Standard and A control test was carried out without sample. Glucose dialysis retardation index (GDRI) was calculated using the following formula:

$$\text{GDRI\%} = \frac{\text{Glucose content with addition of sample (mg/dL)}}{\text{Glucose content of the control (mg/dL)}} \times 100$$

#### Glucose uptake by yeast cells:

Yeast cells were prepared according to the method of Cirillo. Commercial baker's yeast was washed by repeated centrifugation (4200r/minutes,5 minutes) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of extracts (1-5mg) were added to 1ml of glucose solution (5-25mmol/L and incubated together for 10 minutes at 37°C. The reaction was started by adding 100uL of yeast suspension, vortexed, and further incubated at 37°C for 60 minutes. After 60 minutes, the tubes were centrifuged (3800r/minutes, 5 minutes) and glucose was estimated in the supernatant. Gliclazide was used as Standard. The percent increase in glucose uptake by yeast cells was calculated using the following formula:

$$\text{Increase in glucose uptake (\%)} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

**Organoleptic properties**

Size -small,1-inch(2.5cm) ovals

Color -fruit is deep green

Taste -cucumber-like taste

**Extractive yield**

Total ash value of the sample= $100(z-x)/y\%$

$100(62-61)/2=100(1)/2=50\%$

**Microscopical characters:**

Figure 1: Microscopic image of Xylem Vessels



Figure 2: Microscopic Image of Phloem Fibres

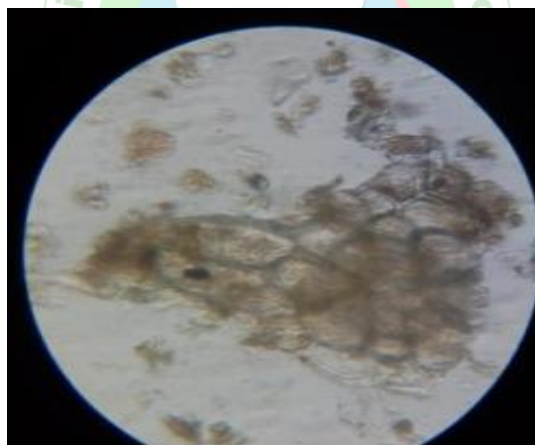


Figure 3: Microscopic Image of Stomata

Table 2: Glucose Adsorption Capacity

Glucose in mmol	STANDARD	EXTRACT	Glucose Bound	
	(Glucose conc mg/dl)	(Glucose conc mg/dl)	Standard	Extract
5 mmol/l	74.3	95.83	10.71	17.44
10 mmol/l	109.75	112	14.5	18.87
20 mmol/l	126.6	126.9	18.67	23.37
50 mmol/l	132.6	133.3	52	54.09
100 mmol/l	133.3	134.4	270	285.6

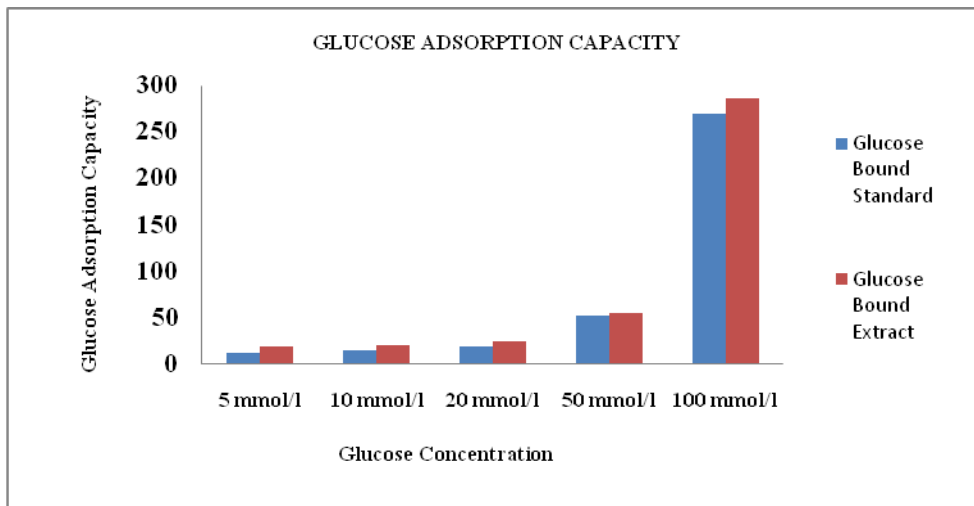


Figure 4: Comparison of Glucose Adsorption Capacity of Standard Drug Gliclazide and Ethanolic extract of Melothria Scabra

Table 3: In vitro Glucose Diffusion Inhibitory Assay

Samples	Glucose Content in dialysate (mg/dl) and GDRI%			
	30 min	60min	120min	180 min
Control	85.8	106.8	128.5	194.6
Standard	72.4 (84.3)	85.3 (79.8)	89.6 (69.7)	112.2 (57.6)
Extract	67.2 (78.3)	78.4 (73.4)	79.6 (61.9)	104.5 (53.6)

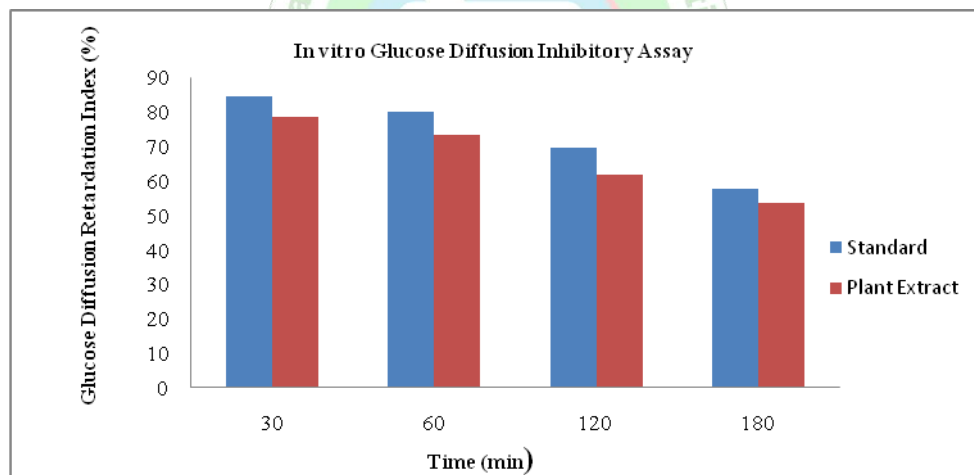
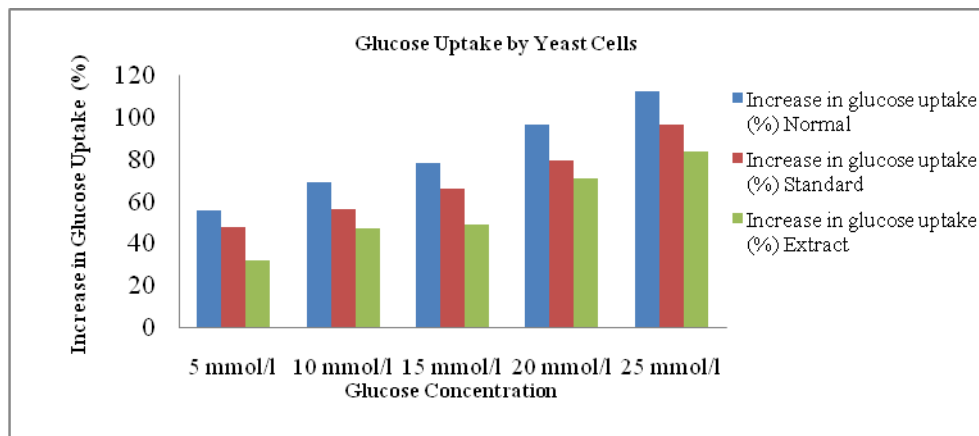


Figure 5: Comparison of In vitro Glucose Diffusion Inhibitory Assay of Standard Drug Gliclazide and Ethanolic extract of Melothria Scabra

Table 4: Glucose Uptake by Yeast cells

Glucose Solution in mmol	Increase in glucose uptake (%)		
	Normal	Standard	Extract
5 mmol/l	56.32	48.09	32.36
10 mmol/l	69.52	56.81	47.76
15 mmol/l	78.48	66.47	49.23
20 mmol/l	96.67	79.82	71.57
25 mmol/l	112.51	96.64	84.21



**Figure 6:** Comparison of Glucose Uptake by Yeast cell between Standard Drug Gliclazide and Ethanolic extract of *Melothria Scabra*

## Discussion

The fruits of *Melothria scabra* are widely used vegetable crop having various health benefits, yet little information can be found in mainstream literature. The current study quantifies the constituent phytochemical groups in the leaves of the plant. In the present study investigation of anti-hyperglycemic potential of *Melothria scabra* was performed by In vitro methods.

Glucose adsorption onto adsorbents is a physical phenomenon, in the intestine the adsorption of glucose onto food matrix can result in decreased free glucose in the solution, thus can reduce the available glucose for uptake by the intestinal epithelial cells.

Enzymes such as  $\alpha$ -amylase,  $\alpha$ -glycosidase, sucrose and lactase play a crucial role in a complete digestion of polysaccharides into simpler monosaccharide units. The liberated monosaccharide diffuse to intestinal epithelial cells where they are taken by the passive diffusion, facilitated diffusion through transporters named Glut transporters and by co-transport with other ions, mainly sodium ion. The process of glucose absorption of any of the above process hindered.

The plant extract has shown good glucose adsorbing and glucose diffusion retardation potential thus indicating its ability to decrease the glucose availability to diffusion into blood stream.

Photochemical synthetic components which retard any of the processes can be considered as anti-hyperglycemic agents and act by inhibiting the entry of glucose into blood stream.

The rate of glucose transport across the cell membrane was studied in an invitro system comprising of yeast cells suspended in different glucose concentration (5-25mmol/L), the percent increase in the glucose uptake by the yeast cells was observed to be inversely proportional to the glucose concentration and was found to be decreased with increase in molar concentration of glucose and plant extract have shown significant decrease in uptake of glucose.

## CONCLUSION

Preliminary assays in the present investigation indicated the anti-diabetic potential of *Melothria scabra*. Hypoglycemic

activity of the plant extract mediated by increasing glucose adsorption, decreasing glucose diffusion rate and glucose transport across the cell membrane. However, these results should be confirmed by in vivo models and further research support is needed to validate the observed potential for effective utilization as therapeutic agents. Nevertheless some of the plants in this family need further study so that new biomolecules can be isolated and identified and ultimately one can develop new phytopharmaceutical agents which may be used as such or as a lead compound for synthesis and modification. The research and development of herbal formulation is highly relevant as it may be less toxic and can be used for mono or co-therapy with other drugs. The ultimate aim of all medical and pharmacological research is to cure diseases to maintain the health of the individual and to improve the quality of life.

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