



## *In Vitro* Antidiabetic Potentials of Methanol Leaf Extract of *Thaumatococcus daniellii* (Miracle Berry)

<sup>1\*</sup>Abdullahi, S.U., <sup>2</sup>Abubakar, Y. G., <sup>3</sup>Akanya, H.O., <sup>3</sup>Abubakar, N.S., <sup>3</sup>Saidu, N.A. and <sup>1</sup>Ahmad, A.

<sup>1</sup>Department of Biochemistry and Molecular Biology, Federal University Dutsin-ma, Nigeria

<sup>2</sup>Department of Biological Sciences, Niger State Polytechnic Zungeru, Nigeria.

<sup>3</sup>Department of Biochemistry, Federal University of Technology Minna, Nigeria

### ABSTRACT

Plants with antioxidant properties have been found useful in the management of diabetes. The plant *Thaumatococcus daniellii* has been used locally in its management. The aim of this study was to evaluate the *in vitro* antidiabetic potentials of methanol leaf extract of *Thaumatococcus daniellii*. The methanol extract of the leaf was obtained and subjected to phytochemical screening. The inhibitory activity of the extract on carbohydrate hydrolyzing enzymes ( $\alpha$ -amylase and  $\alpha$ -glucosidase) and its effect on glucose uptake by yeast cells were determined. The extract was found to contain alkaloids, tannins, flavonoids, saponins, steroids, and phenols. However, it possesses low inhibitory activity on  $\alpha$ -amylase (16 %) and  $\alpha$ -glucosidase (32 %) but increase glucose uptake by yeast cells at 5 mM (86 %), 10 mM (84 %) and 25 mM (72 %) glucose concentration. The methanol leaf extract of *Thaumatococcus daniellii* probably exerts its effect on diabetes through enhancement of glucose uptake.

**Keywords:** Diabetes,  $\alpha$ -amylase,  $\alpha$ -glucosidase, Glucose uptake, *Thaumatococcus daniellii*

### INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism resulting from defects from insulin secretion, insulin action, or both (Rajiv and Sasikumar, 2012). It is as a result of either the pancreas not producing sufficient insulin or the cells of the body not responding properly to the insulin produced (David and Gardner, 2011). The treatment of diabetes mellitus primarily aims at achieving effective control of the elevated blood sugar levels. The available therapies for the control of diabetes mainly target stimulation or enhancement of action of insulin at the target tissues, or modify glucose utilization. Extract from herbs modify glucose utilization by altering the viscosity of gastrointestinal content, delaying gastric emptying or delaying glucose absorption through the inhibition of Carbohydrate hydrolyzing enzymes (Alpha Amylase and Alpha Glucosidase) (Wadkar *et al.*, 2008).

*Thaumatococcus daniellii* (commonly known as miracle berry or African serendipity) belongs to the family *Marantaceae* and native to West Africa, particularly Ghana, Ivory Coast and

Nigeria (Arowosoge and Labode, 2006). It contains a sweetening protein called thumatin (Elemo *et al.*, 2011). Thumatin is a non-toxic intensely sweet protein which is at least 3000 times as sweet as Sucrose. Traditionally, the aril is used for sweetening bread, over-fermented palm wine and sour food. Thumatin is used as a non-caloric sweetener and flavor enhancer by food and confectionary industries. However, it is not a carbohydrate, thus an ideal sweetener for diabetics (Stephen *et al.*, 1994). The leaves on the other hand are used for roofing and wrapping of foods. It is also used as sedative, antidote against venoms, stings and bites and also for treatment of insanity (Kadri *et al.*, 2011). Studies have also shown that it has antidiabetic potentials and also antifungal property and as such can be a natural alternative source of preservative in food industry (Grilo and Lawal, 2010).

Received 08 February, 2021

Accepted 13 April, 2021

Address Correspondence to:

[shehuman88@gmail.com](mailto:shehuman88@gmail.com); +2347039252550

The treatment options available for diabetes, ranging from physical exercise, healthy diet, through Oral medications and Insulin injection often had limited efficacy, risk of adverse effects and are mostly too costly especially for the developing countries like Nigeria (Pallab and Amartya, 2012). Furthermore, among the various plants that are readily used in the treatment of diabetes, only few have undergone scientific validation (Das and Barman, 2012) and these necessitate this study. This study was therefore aims at investigating the *in vitro* antidiabetic potentials of methanol leaf extract of *Thaumatococcus danielli*.

## METHODOLOGY

### Enzymes

Alpha-amylase from *Aspergillus Oryzae* was a product of Sigma-Adrich Co., St Louis, USA, and Alpha Glucosidase was obtained from rat intestine

### Isolation of $\alpha$ -glucosidase enzyme

Two (2) albino rats were sacrificed, intestine removed and chilled with ice cold 80 mM phosphate buffer pH 6.9. The intestine was then cut open, the mucosa was scraped with a piece of glass rod and homogenized with four parts (v/v) of cold buffer. Nuclei and large cell debris were removed by centrifugation at 2000 rpm for 10 mins and the supernatant was divided into 1.5 ml vials and stored at -20 °C.

### Plant Sample

The fresh leaves of *Thaumatococcus danielli* plant were collected from Wawagi village in Lavun local government area of Niger State and were presented to a Botanist in Biological Science Department, Federal University of Technology Minna for identification.

### Preparation of Crude Extract

The leaves collected was thoroughly washed with water and dried at room temperature. The dried leaves were pulverized into coarse powder using an electric blender. The partially powdered sample was extracted by hot decoction using methanol at 70 °C for two hours in a reflux extractor. This was then evaporated to dryness in a water bath at 40 °C. The gummy extract obtained was weighed and stored in the refrigerator at 4 °C for further analysis.

## Qualitative Phytochemical Analysis

The method described by Trease and Evans (1999) was employed for the qualitative phytochemical analysis.

### Alpha amylase inhibition assay

The effect of methanol leaf extract of *Thaumatococcus danielli* on alpha amylase inhibition was determined using the procedure reported by (Keerthana *et al.*, 2013). The enzyme solution was prepared by dissolving 1 mg of alpha-amylase in 100 ml of 20 mM phosphate buffer (pH 6.9). The different concentrations of the extract (0.2, 0.4, 0.6, 0.8 and 100 mg) were prepared in DMSO. Starch solution (0.5 % w/v) was used as substrate and was prepared by boiling potato starch in distilled water for 15 min. 0.5 ml of the plant sample was mixed with 0.5 ml of enzyme solution and the mixture was incubated at 25 °C for 30 min. This was then followed by addition of 1 ml of the starch solution; and the mixture was again incubated at 25 °C for 3 min. 1 ml of the DNSA solution was added and the mixture was heated in a water bath at 85 °C for 15 min. After cooling, the mixture was diluted with 9 ml of distilled water. It was thoroughly mixed and the absorbance was recorded at 540 nm using UV-spectrophotometer. For each concentration, Individual blanks were prepared for correcting the background absorbance by replacing the enzyme solution with buffer; the DNS solution was added before the addition of starch solution. For the control, the plant extract was replaced with the same volume of DMSO. Acarbose was used as standard. The  $\alpha$ -amylase inhibitory activity was calculated using the following formula:

$$\text{The } \alpha\text{-amylase inhibitory activity (\%)} = \frac{[(Ac+) - (Ac-)] - [(As-Ab)]}{[(Ac+) - (Ac-)]} \times 100$$

Ac+ = the absorbance of 100% enzyme activity (only solvent with enzyme),

Ac- = the absorbance of 0% enzyme activity (only solvent without enzyme)

As = the absorbance of a test sample (with enzyme)

Ab = absorbance of a blank (a test sample without enzyme).

### Alpha-glucosidase inhibition assay

Alpha-glucosidase activity was measured *in vitro* by determination of the reducing sugar (glucose) arising from hydrolysis of sucrose by

$\alpha$ -glucosidase enzyme, isolated from small intestine of rat. 225  $\mu$ l of 80 mM phosphate buffer, pH 7.0 was added to different concentrations (200, 400, 600, 800 and 1000  $\mu$ g/ml) of the plant sample followed by addition of 75  $\mu$ l of the glucosidase and was pre-incubated at 37 °C for 30 min. 500  $\mu$ l of 37 mM sucrose was then added to the mixture and was also incubated at 37 °C for 20 minutes. The solution was kept in boiling water bath for 2 minutes to stop the reaction and then cooled. 250  $\mu$ l of glucose reagent was added and incubated at room temperature for 10 minutes after which the absorbance was read at 510 nm using the UV-spectrophotometer. Acarbose was used as the standard. The analysis was carried out in triplicate (Matsui *et al.*, 2007).

Glucosidase inhibition (%) =

$$\frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \times 100$$

### Glucose uptake by yeast cells assay

Commercial baker's yeast (*Saccharomyces Cerevisiae*) was washed by repeated centrifugation (4200 r/min, 5 min) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of extracts (200, 400, 600, 800 and 1000  $\mu$ g/ml) were added to 1 ml of glucose solutions (5, 10 and 25 mM) and incubated together for 10 min at 37 °C. The reaction was started by adding 100  $\mu$ l of yeast suspension, vortexed and further incubated at 37 °C for 60 min. After 60 min, the tubes were centrifuged (3800 r/min, 5 min) and glucose was estimated in the supernatant (Sindhu, *et al.*, 2013). Metformin was used as the standard. The percentage increase in glucose uptake by yeast cells was calculated using the following formula:

Glucose uptake (%) =

$$\frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \times 100$$

### Statistical analysis

Values were analyzed using statistical package for social science (SPSS) version 16 and

presented as means  $\pm$  standard error of the mean. Comparisons between different groups were carried out by one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The level of significance was set at  $P < 0.05$ .

## RESULTS

There was significant ( $p < 0.05$ ) increase in the inhibitory activity of Acarbose (Standard diabetic drug) on alpha amylase (Figure 1) and alpha glucosidase (Figure 2) with increase in concentrations. However, there was no significant ( $p > 0.05$ ) increase in the inhibitory activity of the plant extract on alpha amylase and alpha glucosidase with increase in concentrations. Furthermore, There was significant ( $p < 0.05$ ) increase in glucose uptake by yeast cells at 5 mM, 10 mM and 25mM glucose concentration with increase in the concentration of the plant extract and Metformin (Figure 3, 4, 5). At 5mM glucose concentration (Figure 3), the plant extract showed  $IC_{50}$  value of 181  $\mu$ g/ml and Metformin (standard) 84  $\mu$ g/ml, while at 10 mM glucose concentration (Figure 4), the extract showed  $IC_{50}$  value of 286  $\mu$ g/ml and Metformin 193  $\mu$ g/ml. The extract showed  $IC_{50}$  value of 538  $\mu$ g/ml and Metformin (standard) 475  $\mu$ g/ml at 25 mM glucose concentration (Figure 5)

**Table 1:** Qualitative Phytochemical Constituents of methanol leaf extract of *Thaumatococcus daniellii*

Phytochemicals	Observation
Alkaloids	+
Flavonoids	+
Phenols	+
Tannins	+
Saponins	-
Steroids	+
Terpenes	-
Cardiac glycosides	+
Anthraquinone	-
Present	+
Absent	-

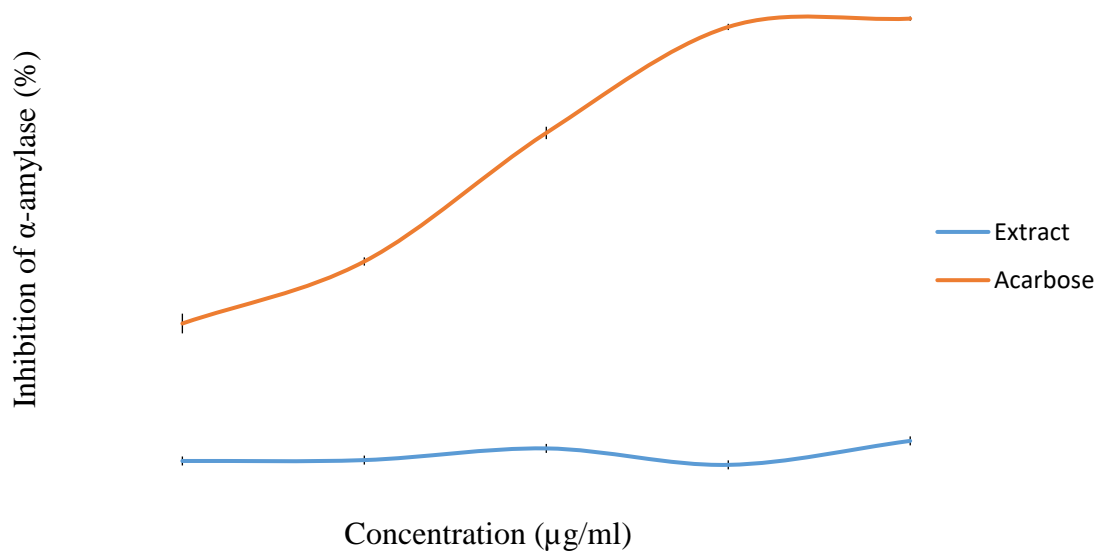


Figure 1: Inhibitory Activity of the Methanol Leaf Extract of *Thaumatococcus daniellii* and Acarbose (Standard diabetic drug) on Alpha Amylase

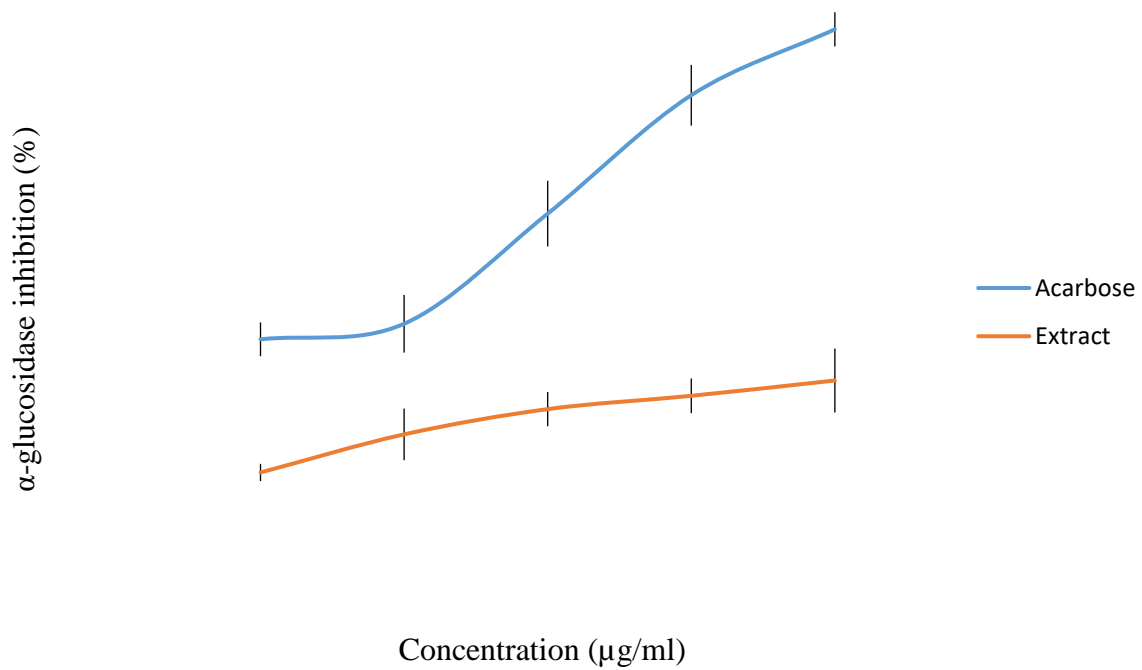


Figure 2: Inhibitory Activity of the Methanol Leaf Extract of *Thaumatococcus daniellii* and Acarbose (Standard diabetic drug) on Alpha Glucosidase

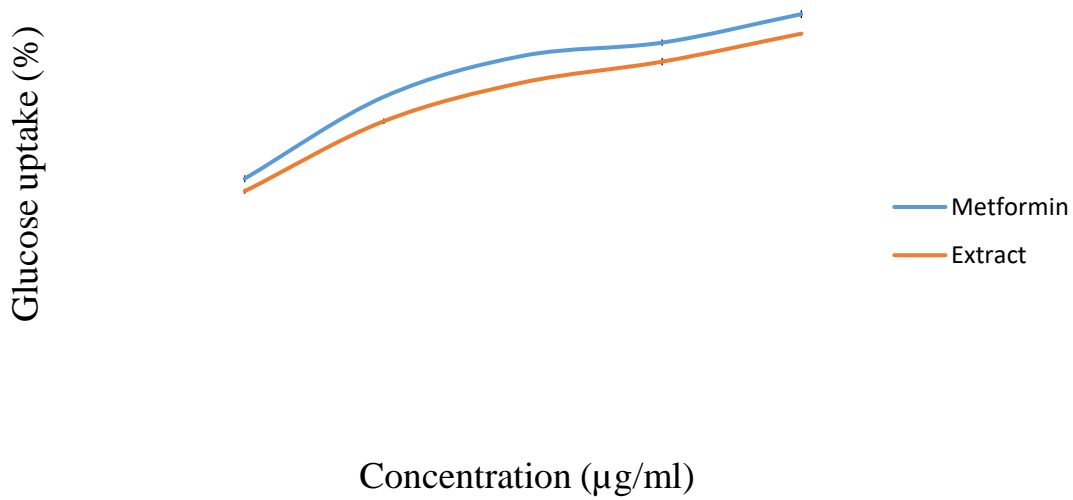


Figure 3: Effect of Methanol Leaf Extract of *Thaumatococcus daniellii* and Metformin (standard drug) on Glucose Uptake by Yeast Cells at 5 mM Glucose Concentration.

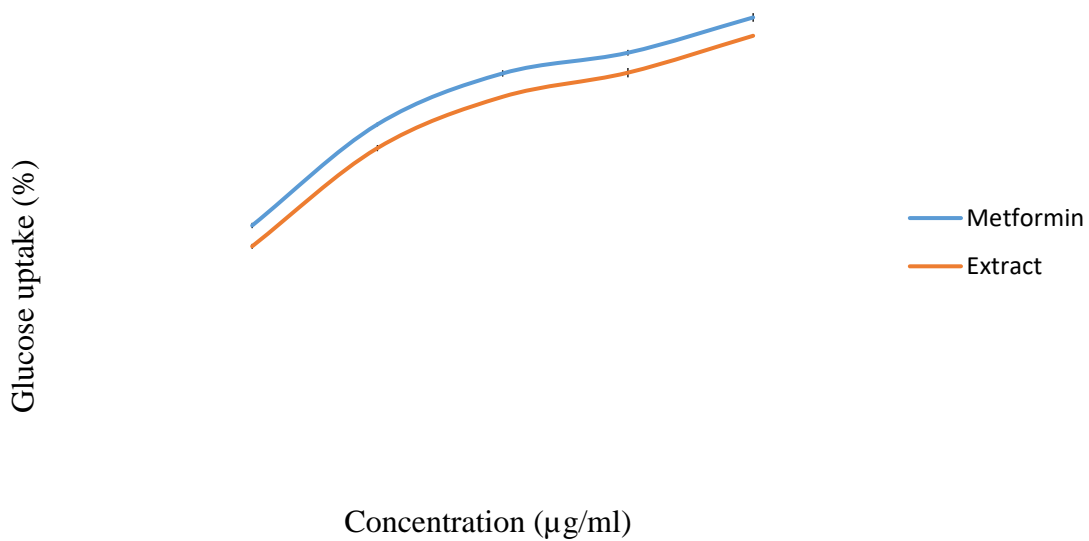


Figure 4: Effect of Methanol Leaf Extract of *Thaumatococcus daniellii* and Metformin (standard drug) on Glucose Uptake by Yeast Cells at 10 mM Glucose Concentration.

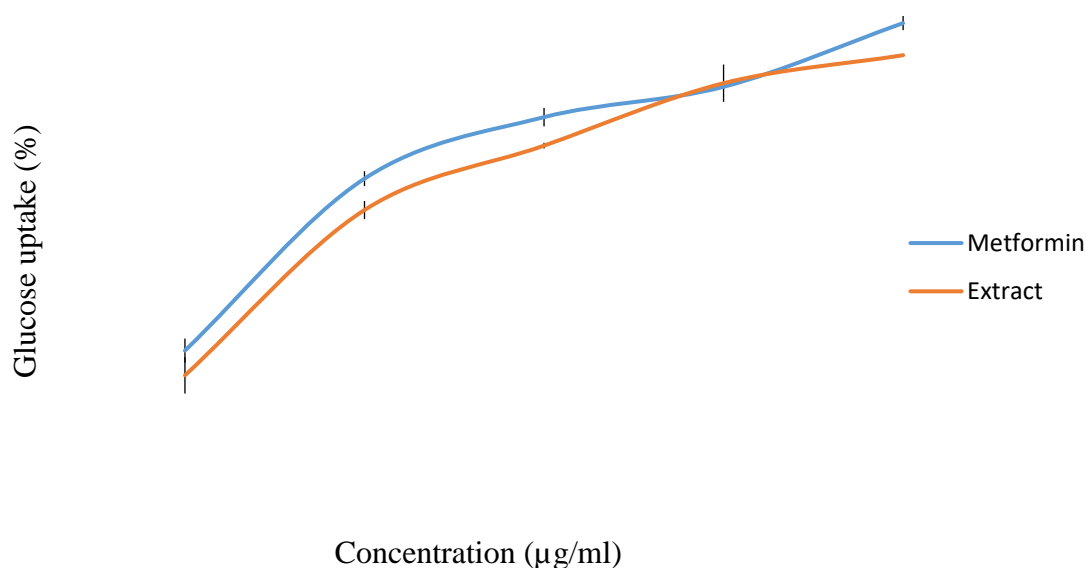


Figure 5: Effect of Methanol Leaf Extract of *Thaumatococcus daniellii* and Metformin (standard drug) on Glucose Uptake by Yeast Cells at 25 mM Glucose Concentration

## DISCUSSION

The qualitative analyses of the plant studied, revealed the presence of pharmacologically active phytochemicals such as alkaloids, flavonoids, phenols, tannins, steroids and glycosides. The presence of these various phytoconstituents as shown in Table 1 could be responsible for its versatile medicinal properties. This is in agreement with the previously reported study by Oke *et al.*, (2016) and Hamid *et al.*, (2017) on the phytochemical analysis of the same plant.

The low percentage inhibition of alpha amylase by the plant extract in the present study (Figure 1) signifies its inability to effectively inhibit  $\alpha$ -amylase activity. This is also in agreement with the previously reported studies on several plant extracts that have been shown to possess high antidiabetic potentials and lesser ability to inhibit  $\alpha$ -amylase. Extract from *Phyllanthus spp* was previously reported to inhibit  $\alpha$ -amylase activity by 24 % (Ali *et al.*, 2006). Also, oil isolated from *Momordica charantia* exhibited 38 % inhibition of  $\alpha$ -amylase (Zuraini *et al.*, 2012). Upon the low percentage inhibition of the enzyme, these plants still exert antidiabetic activity by stimulating insulin release or suppressing glucose production by the liver. The low inhibitory activity of the plant extract on

alpha glucosidase obtained in the present study (Figure 2) is also in agreement with the previously reported study by Babu *et al.*,(2004) that showed 35 % inhibition of Himalayan herbs constituents that are used in treating diabetes. Therefore, the low inhibition of alpha amylase and alpha glucosidase in this study (Figures, 1 and 2) suggests that the plant extract might lack the ability of being a potent inhibitor of  $\alpha$ -amylase and  $\alpha$ -glucosidase, however, may still exert antidiabetic activity through other mechanisms.

The glucose uptake rate increased with increasing concentration of the plant extract at the various glucose concentrations and decreased with increasing extracellular glucose concentration (Figures 3, 4 and 5). This can be attributed to the fact that the transport of glucose across the yeast cell membrane occurs by facilitated diffusion down the concentration gradient and glucose transport across the membrane occurs only if the intracellular glucose is effectively utilized (Ahmed *et al.*, 2009). The significant increase in glucose uptake by yeast cells at 5 mM (86 %), 10 mM (84 %) and 25 mM (72 %) glucose concentration obtained in this present study is also in agreement with the previous study reported by Gupta *et al.*,(2012) on *in vitro* antidiabetic

activity of stem bark of *Bauhinia purpurea* Linn which showed a low (59.23 %) increase in glucose uptake. It has been shown that insulin stimulates glucose metabolism in its target tissue via recruitment of transporters from a large intracellular pool to the plasma membrane and the functional activity of the transporters are impaired in diabetic state (Bitar *et al.*, 2005). It was also reported by Jorge *et al.*, 2004 that Kaempferitin (flavonoid glycoside isolated from *Bauhinia forficata*) showed blood lowering activity through altered intrinsic activity of glucose transporter. These suggest that the increase in glucose uptake upon treatment of yeast cells with various concentrations of plant extract might be as a result of stimulating effect exerted on glucose transporter in the plasma membrane thereby enhancing glucose uptake and consequently reduce blood glucose level.

### Conclusion

The plant extract has low ability to inhibit  $\alpha$ -amylase and  $\alpha$ -glucosidase activity, hence, may not interfere with carbohydrate digestion, however, the regulation of blood glucose by the extract may be mediated through stimulation of glucose transporters.

### REFERENCES

- Ali, H., Houghton, P. J., & Soumyanath A (2006). “  $\alpha$ -amylase inhibitory activity of some Malaysian plants used to treat diabetes, with reference to *Phyllanthus amarus*” *Journal of Ethnopharmacology*. 107, 449-455.
- Arowosoge, O.G. & Labode, E. (2006). Economic analysis of *Thaumatococcus danielli* (Benn.) Benth. (Miraculous berry) in Ekiti State, Nigeria. *Journal of Food Agriculture and Environment*. 4, 1, 264-269.
- Babu, P. V., Liu, D., & Gilbert, E. R (2013). [Recent advances in understanding the anti-diabetic actions of dietary flavonoids](#). *The Journal of Nutritional Biochemistry*. 24, 11, 1777-1789.
- Bitar, A. A., Todd, P. S., Kaplan, M. D. & Andrew, N. V (2005). Rehabilitation of Orthopedic and Rheumatologic Disorders. *Elsevier*, 86, 1, 48-55.
- Das, S., & Barman, S (2012). Antidiabetic and Antihyperlipidemic effect of ethanolic extract of leaves of *Punica Granatum* in alloxan-induced non-insulin-dependent Diabetes mellitus albino rats. *Indian Journal of Pharmacology*. 44, 219-224.
- David, G., W & Gardner, D. (2011). *Greenspan's Basic and Chemical Endocrinology*, (9<sup>th</sup> edition) New York: McGraw-Hill medical, 17, 187.
- Elemo, B.O., Adu, O.B., Ogunrinola, O.O., Efuwape, T.O., Olaleye, K. O. & Kareem A.A. (2011). Biological Evaluation of *Thaumatococcus danielli* Waste Protein. *Pakistan Journal of Nutrition*. 10, 1048-1052.
- Grillo, J.A. & Lawal, A. K. (2010). *In vitro* activity of *Thaumatococcus daniellii* and *Megaphrynium macrostachyum* against spoilage fungi of white bread and Eba' an indigenous staple food in Southern Nigeria. *African Journal of Microbiology Research*. 4, 1076-1081.
- Gupta, D., Kondongala, S., & Chandrashekhher, G. (2012). *In vitro* antidiabetic activity of stem bark of *Bauhinia purpurea* Linn. *International Journal of Pharmacology*, 2, 2277-3436.
- Hamid, A. A., Aliyu, M. A., Abubakar, L. Z., Mukadam, A.A., Shehu, A., Egharevba & Adisa, M. J (2017). *Thaumatococcus danielli* leaves: Its Composition, Antioxidant and Antimicrobial activities. *Ife Journal of Science* 19 (2), 409-416.
- Jorge, A. P., Horst, H., de Sousa, E., Pizzolatti, M. G., & Silva, F. R. (2004). Insulinomimetic effects of kaempferitrin on glycaemia and on <sup>14</sup>C-glucose uptake in rat soleus muscle. *Chemistry-Biology Interaction*, 149, 89-96.
- Kadri, A., Zarai, Z., Békir, A., Gharsallah, N., Damak, M & Gdoura, R. (2011). Chemical composition and Antioxidant activity of *Marrubium Vulgare* L. essential oil from Tunisia. *African Journal of Biotechnology*, 10, 19, 3908-3914.
- Keerthana, G., Kalavani, M. K., & Sumathy, A (2013). *In vitro* alpha amylase inhibitory and antioxidant activities of ethanolic leaf extract of *Croton bonplandianum*. *Asian Journal of Pharmacy and Clinical Resilience*, 3, 32-36.
- Matsui, T., Tanaka, T., Tamura, S., Toshima, A., Miyata, Y., & Tanaka, K (2007). Alpha glucosidase inhibitory profile of catechins and

- theaflavins. *Journal of Agriculture and Food Chemistry*, 55, 99-105.
- Oke, I. A., Olufemi, A., Oluwagbenga, D., Olaoluwa, A., & Godswill, N.A (2016). Phytochemical Constituent and Antioxidant activity of *Thaumatococcus danielli* Benn (*Benth*) leaves (food wrapper). *International Journal of Pharmacology, Phytochemistry and Ethnomedicine*, 2, 55-61.
- Pallab, D. G., & Amartya, D. E (2012). Diabetes mellitus and its herbal treatment. *International Journal of research in pharmaceutical and Biomedical Sciences*, 3, 2, 717-718.
- Rajiv, G, G. & Sasikumar, P. (2012). Antidiabetic effect of *Merremia marginata* Burm. F. in streptozotocin induced diabetic rats. *Asian Pacific Journal of Tropical Biomedicine*, 2, 281-286.
- Sindhu, S. N., Vaibhav, K & Anshi, M (2013). Evaluation of *in vitro* antidiabetic activity of selected plant extracts. *International Journal of Pharmaceutical Science Invention*, 2, 4, 12-19.
- Stephen, A. G., Powls, R. & Beynon, R. J., (1994). Cysteine protease activity in arils of *Thaumatococcus daniellii*: Relationship between the sweet protein thaumatin and cysteine protease activity. *International Journal of Biochemistry*. 26, 8798-88.
- Trease G. E., & Evans, W.C (1989). *Pharmacognosy*, 11<sup>th</sup> Edition, Bailliere Tindal, London, 45-50
- Wadker, K. A., Magdum, C. S., Patil, S. S., & Nalkwade, N. S (2008). Antidiabetic potential and Indian medicinal plants. *Journal of Herbal Medicine and Toxicology*, 2, 45-50.
- Zuraini, A., Somchit, M. N., Solihah, M. H., Arifah, A. K., Zakaria, M. S., Somchit N., .... & Mait, A. M (2006) "Fatty acid and amino acid composition of three local Malaysian *Channa* spp. *Fish Food Chemistry* 97, 674-678