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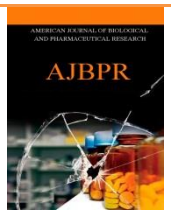

DEVELOPMENT AND PHARMACOLOGICAL EVALUATION OF HERBAL DOSAGE FORM FOR ANTIDIABETIC ACTIVITY

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ABSTRACT

The main objective of this work is to evaluate the in vitro anti diabetic activity of methanolic extracts of *Manilkara zapota*. The selected plant extracts were studied for their effect on inhibition of glycosylation of haemoglobin and glucose transport across yeast cells. It was found that the percentage increase of the rate of uptake of glucose into yeast cells was linear in different glucose concentrations used. *Manilkara zapota* showing the maximum increase in10 mM Glucose concentration i.e. 78.42 % at 2000 µg/mL. Glycosylated hemoglobin is measured primarily to identify the average plasma glucose concentration over prolonged periods of time. In Diabetes mellitus higher amounts of glycated hemoglobin indicates poor control of blood glucose levels. The methanolic extracts of the plants also showed a considerable inhibition of the haemoglobin glycosylation as compared to standard gallic acid. The results of the work indicate that the selected plants possessed considerable In vitro anti diabetic activity and further these effects need to be confirmed using in vivo models for its effective utilization as therapeutic agents.

INTRODUCTION

Plants have served mankind since ages as they are reservoirs of important medicinal components and help to alleviate chronic diseases. The past was considered the synthetic era due to the commercial production of large varieties of synthetic drugs by pharmaceutical industries [1]. Over time the continuous use of synthetic drugs caused severe side effects, and led to resistance of microbes. Also synthetic drugs are expensive and large populations cannot afford to get benefit from these drugs. During the last decades a global trend with focus on green medicines due to minimum side effects and cost effectiveness. Medicinal plants play an appreciable role in the development of modern herbal medicines as many diseases like cancer, liver diseases and arthritis find no complete cure in allopathy.

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The bioactive compounds of medicinal plants are used as antidiabetic, chemotherapeutic, anti inflammatory, anti arthritic agents where no satisfactory cure is present in modern medicines [2]. Many plants have shown their immense potential to fight against dreadful diseases including cancer. Diabetes mellitus is a chronic endocrine disorder that affects the metabolism of carbohydrates, proteins, fat, electrolytes and water and includes a group of metabolic diseases characterized by hyperglycemia. Currently, there is growing interest in herbal remedies due to the side effects associated with the oral hypoglycemic agents for the treatment of *diabetes mellitus* [3]. Hence the traditional herbal medicines are mainly obtained from plants are used in the management of diabetes mellitus. In recent years herbal medicines have started to gain importance as a source of hypoglycemic agents. It is estimated that more than thousand plant species are being used as folk medicine for diabetes [4]. Biological actions of the plant products

used as alternative medicines to treat diabetes are in relevance to their chemical composition. Herbal products or plant products are rich in flavonoids, phenolic compounds, coumarins, terpenoids and other constituents which help to reduce blood glucose levels [5]. Several species of herbal drugs with potential anti-diabetic activity have been described in the scientific literature. Herbal drugs are prescribed due to their good effectiveness, fewer side effects in clinical experience and relatively low costs [6]. Medicinal and natural herbal plant products are traditionally used from long time in many countries for the treatment of diabetes mellitus. The present work was to evaluate the in vitro anti diabetic activity of methanolic extracts of *Manilkara zapota* by studying their effects on inhibition of glycosylation of haemoglobin and glucose transport across yeast cells.

MATERIALS AND METHODS

Collection and preparation of plant extracts

Leaves of *Manilkara zapota* was obtained from Jhansi, UP. The plant parts were washed with distilled water. The collected plant material was air dried in darkness at room temperature. Dried plant parts were cut into small pieces and blended into uniform dry powder. About 10g of plant powder was mixed with 100 mL of methanol solvent. The mixture was kept on the rotary shaker for 72 hrs. Extract was filtered through Whatmann No.1 filter paper and centrifuged at 5000 g for 15 minutes and the supernatant was collected and concentrated using rotary evaporator. The concentrated plant extract was stored at 0-4°C for further use [7].

Glucose uptake inYeast cells

The commercial baker's yeast in distilled water was subjected to repeated centrifugation (3,000×g, 5min) until clear supernatant fluids were obtained and a 10% (v/v) of the suspension was prepared in distilled water. Various concentrations of plant extracts (50- 2000 µg/mL) were added to 1 mL of glucose solution (5, 10 and 25 mM) and incubated together for 10 min at 37 °C. Reaction was started by adding 100 µL of yeast suspension followed by vortexing and further incubation at 37 °C for 60 min. After 60 min, the tubes were centrifuged (2,500×g, 5 min) and amount of glucose was estimated in the supernatant [8]. Metronidazole was used as standard drug. The percentage increasing glucose uptake by yeast cells was calculated using the following formula:

Increasing glucose uptake (%) = Abs sample–Abs control X 100

Abs sample

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. All the experiments were carried out in triplicates.

Evaluation of haemoglobin glycosylation

Preparation of haemoglobin

The blood was collected from a healthy human volunteer and transferred into a blood bottle containing an anticoagulant. Hemolysate was prepared based on the principle of hypotonic lysis [9]. The red blood collected were washed thrice with 0.14 M NaCl solution and one volume of red blood cells suspension was lysed with two volumes of 0.01M phosphate buffer, pH 7.4 and 0.5 volume of CCl4. The haemolysate was then freed from the debris by centrifugation at 2300 rpm for 15 min at room temperature. The haemoglobin rich fraction ie the upper layer was separated and dispensed into sample bottle for storage and refrigerated until required for use [9].

Estimation of haemoglobin glycosylation

1 mL each of haemoglobin fraction was transferred into three test tubes, each containing 1 mL solution of different concentrations (2, 10, and 20 mg/mL) of glucose in 0.01M phosphate buffer (pH 7.4). The contents were incubated at room temperature for 72 hrs. A blank solution in which the addition of glucose solution was omitted was used as the control. The amounts of hydroxyl methyl furfural in nano mole released were estimated at different incubation periods of 0, 24 hrs, 48 hrs and 72 hrs which correspond to the degree of glycosylation [9].

Effect of Extracts on Haemoglobin Glycosylation

To 1 mL of haemoglobin solution, 5 µL of gentamycin and 25 µL of the plant extracts (30µg/mL) were added. The reaction was started by the addition of 1 mL of 2% glucose in 0.01M phosphate buffer (pH 7.4) and incubated in the dark at room temperature. The concentrations of glycated haemoglobin at the incubation period of 0, 24 and 72 hrs were estimated spectophotometrically at 443 nm [9].

Effect of extract at physiological glucose concentration

To 1 mL of haemoglobin solution, 1 mL of glucose solution (2 mg, 10 mg and 20 mg in 20 mL each of 0.01M phosphate buffer, pH 7.4) and 5µL of gentamycin in 0.01M phosphate buffer (pH 7.4) were mixed and incubated in the dark at room temperature in the presence 30 µg/mL of Gallic acid and plant extracts respectively. Haemoglobin concentrations were estimated over an incubation period of 72 hrs spectrophotometrically at 443 nm, as an index for measuring the degree of haemoglobin glycosylation. The assay was carried out in triplicates [9].

Table 1: The comparative effect of plant extracts on haemoglobin glycosylation at physiological glucose concentration after 48 hrs of incubation

	Absorbance at 443 nm after 48 hrs of incubation period				
Glucose concentration	Standard (Gallic acid)	*Manilkara zapota*	*A. heterophyllus*	*C. zeylanicum*	*Pipe rbetle*
2mg/mL	1.019±0.13	0.645±0.18	1.11±0.17	0.293±0.09	0.704±0.08
10mg/mL	1.128±0.11	0.843±0.08	1.211±0.11	0.326±0.16	0.851±0.10
20mg/mL	1.24±0.09	1.11±0.13	1.367±0.14	0.527±0.12	0.95±0.19

Figure 1: The comparative % increase in glucose uptake by yeast cells due to the effect of methanolic extracts of *Manilkara zapota* and reference Standard drug (Metronidazole) at 25 mM Glucose Concentration (values are expressed as mean±SD, n=3)

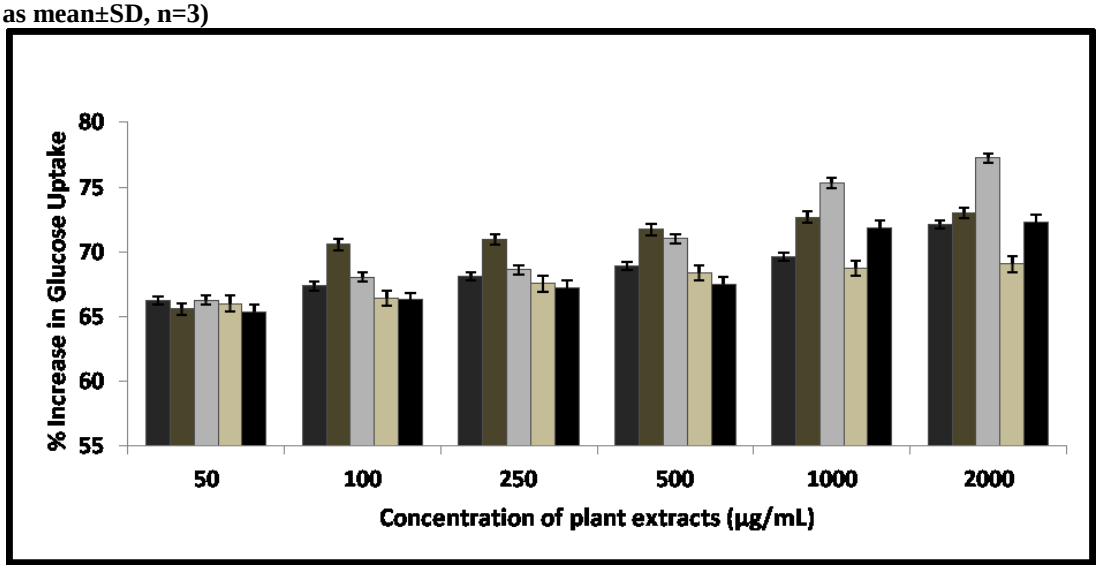


Figure 2: The comparative % increase in glucose uptake by yeast cell due to the effect of methanolic extracts of A. altilis, A. heterophyllus, C. zeylanicum and Piper betle and reference Standard drug (Metronidazole) at 10mM Glucose Concentration (values are expressed as mean ± SD, n=3)

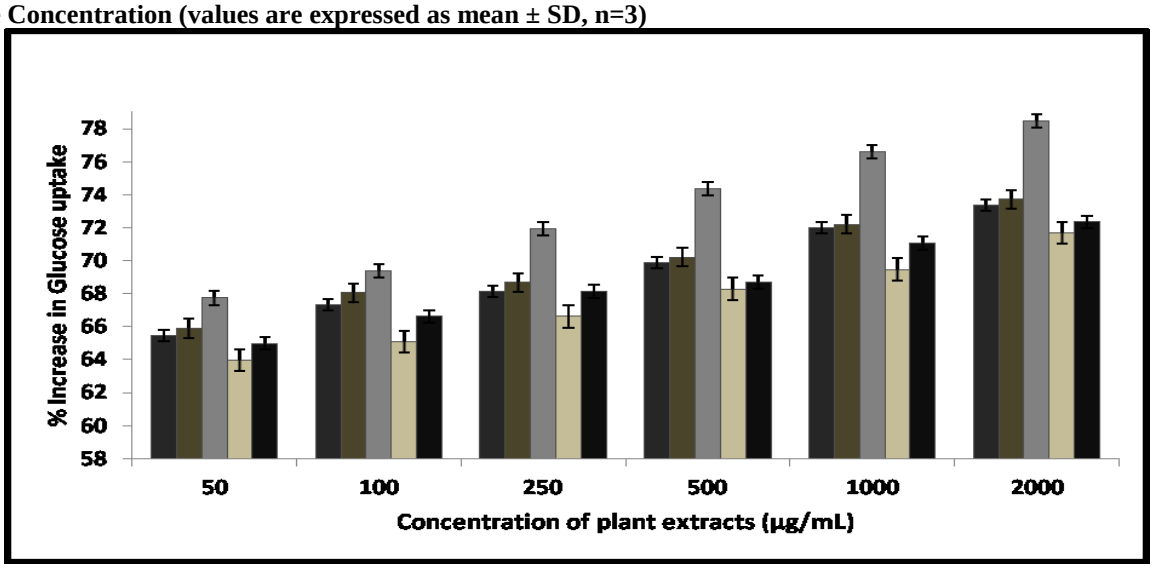


Figure 3: The comparative % increase in glucose uptake by yeast cell due to the effect of methanolic extracts of Manilkara zapota and reference Standard drug (Metronidazole) at 5mM Glucose Concentration (values are expressed as mean±SD, n=3)

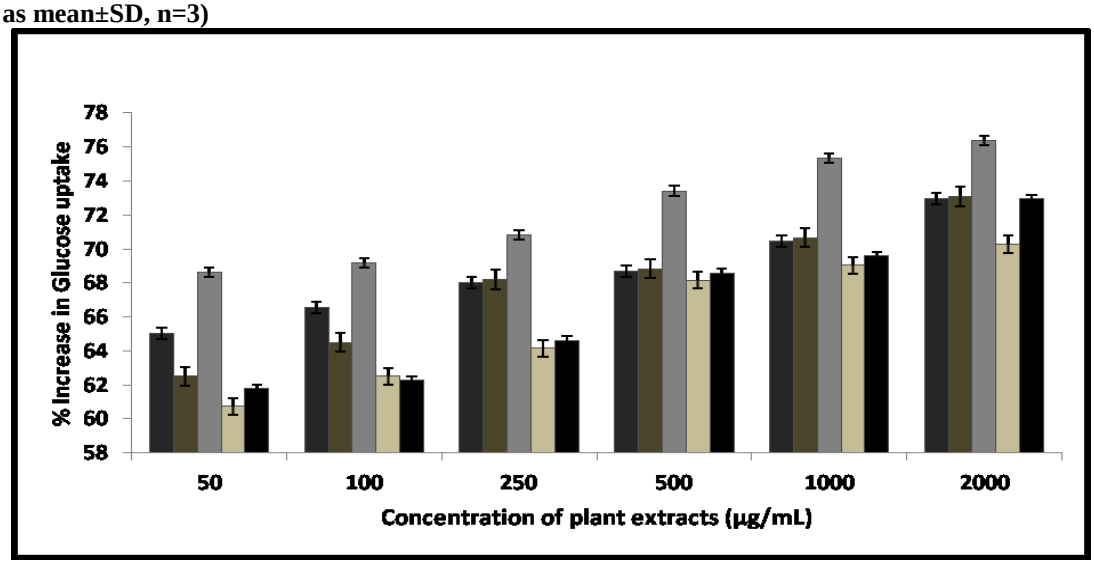


Figure 4: Estimation of haemoglobin glycosylation over the Period of 72 hours (values are expressed asmean±SD, n=3)

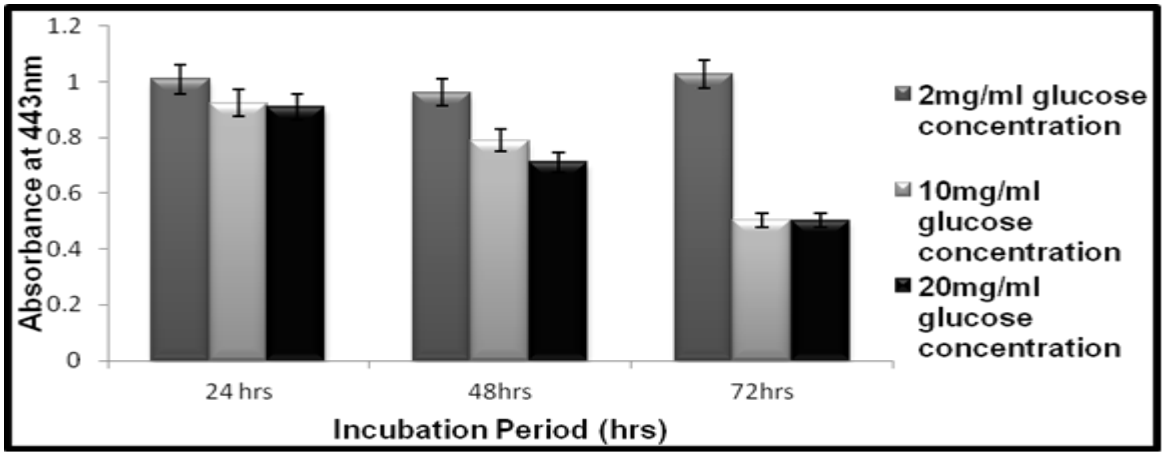


Figure5: Effect of plant extracts on haemoglobin glycosylation (Values are expressed as mean±SD, n=3)

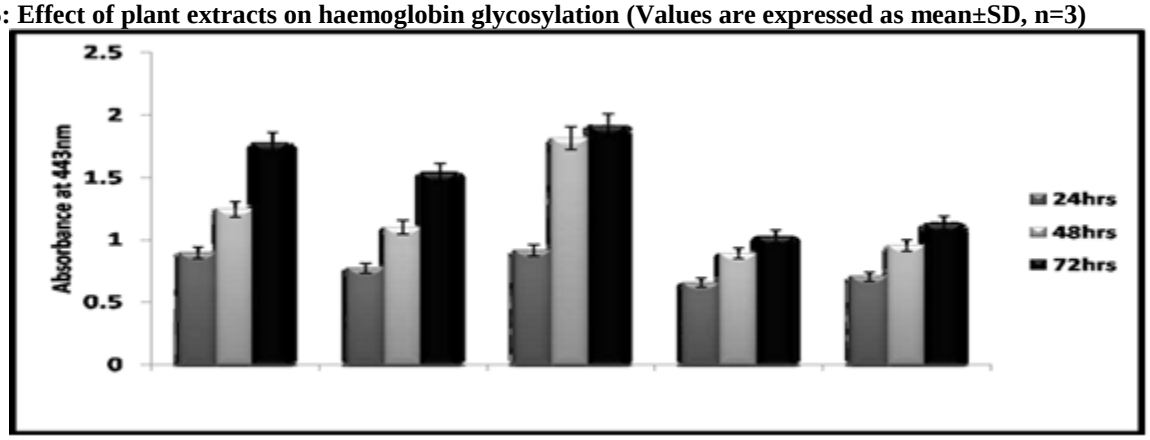


Figure 6: The comparative effects of plant extracts on haemoglobin glycosylation at physiological glucose concentration after 24 hrs of incubation (values are expressed as mean±SD, n=3)

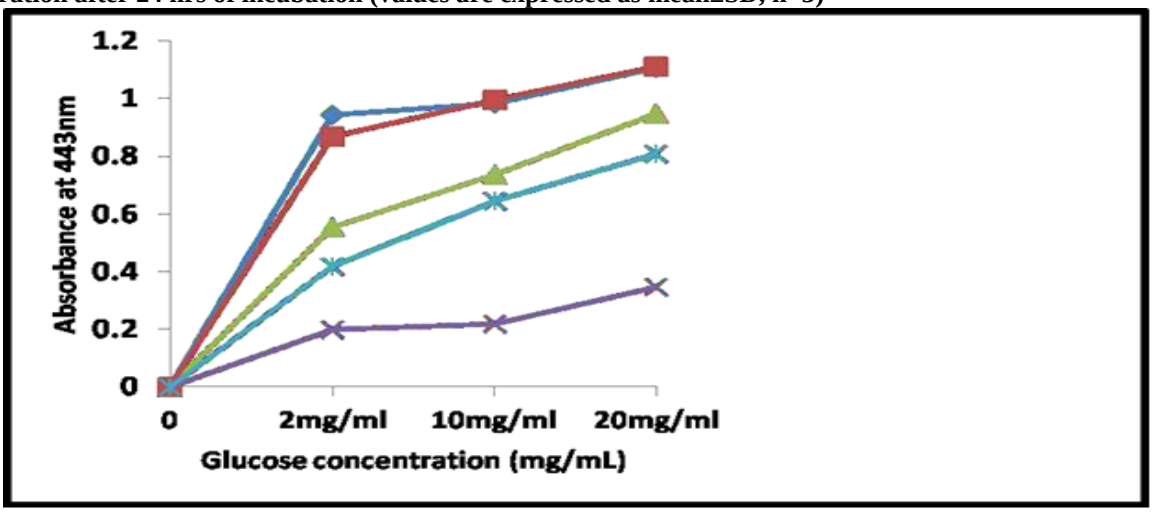


Figure 7: The comparative effect of plant extracts on haemoglobin glycosylation at physiological glucose concentration after 48 hrs of incubation (values are expressed as mean±SD, n=3)

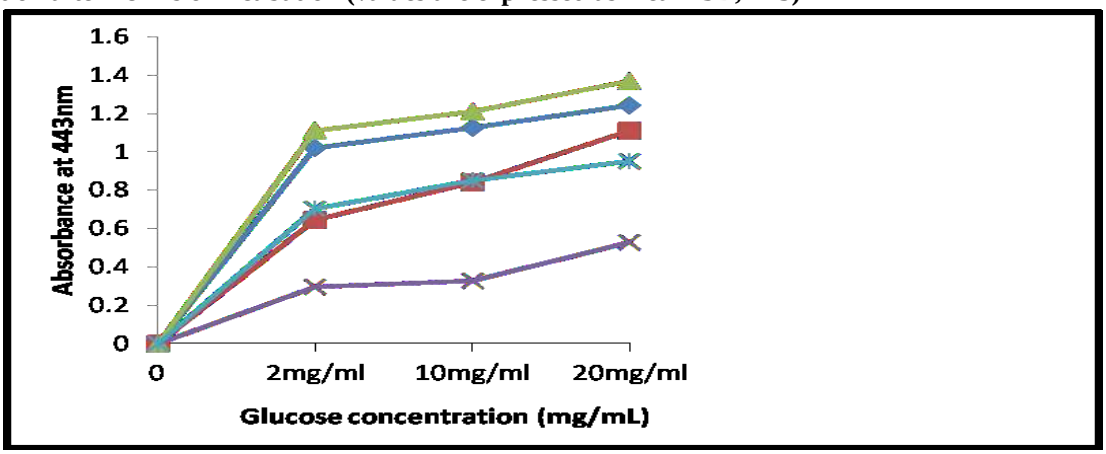
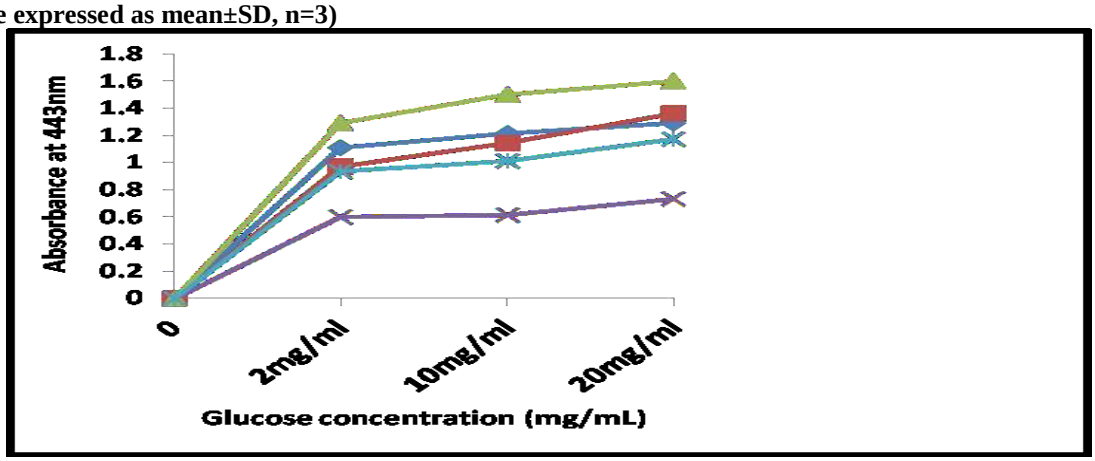


Figure 8: The comparative effects of plant extracts at physiological glucose concentration after72 hrs of incubation (values are expressed as mean±SD, n=3)



RESULTS AND DISCUSSION

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 [10]. The in vitro assays of the present study indicated that all the four plants; *Manilkar azapota* possess good anti-diabetic activity. In Yeast (*Saccharomyces cerevisiae*) glucose transport takes place through facilitated diffusion. Type 2 Diabetes is characterized by the deficiency of insulin causing increased amount of glucose in blood. After the treatment of the yeast cells with the methanolic plant extracts, the glucose uptake was found to increase in a dose dependent manner. Figures 1, 2 and 3 depict the % increase in glucose uptake by the yeast cell at different glucose concentrations i.e. 25 mM, 10 mM and 5mM respectively. The methanolic extracts of *Manilkar azapota* exhibited significantly higher activity than other plant extracts at all glucose concentrations showing the maximum increase in 10 mM Glucose concentration i.e. 78.42% increase at 2000µg/mL of plant extract (Figure 2). Results also indicated that *Manilkar azapota* had greater efficiency in increasing the glucose uptake by yeast cells as compared to standard drug metronidazole.

Increased concentration of glucose in the blood leads to its binding to hemoglobin which may result in the formation of the reactive oxygen species. Plant extracts play an important role the inhibition of the glycosylation end products. An increase in the glycosylation was observed on incubation of hemoglobin with the increasing concentration of the glucose (2 mg, 10 mg and 20 mg) over a period of 72 hrs (Figure 4). However, the plant extracts significantly inhibited the haemoglobin glycosylation which is indicated by the presence of increasing concentration of haemoglobin (Figure 5). *Manilkara zapota* exhibited higher inhibition of glycosylation as compared with the standard gallic acid. The plant extracts also displayed the inhibition of haemoglobin glycosylation at different physiological concentrations of the glucose over the period of 72 hrs, indicating that the plant extracts decreases the formation of the glucose-haemoglobin complex and thus amount of free haemoglobin increases (Figures 6, 7 and 8).

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The results indicate that methanolic extracts of *Manilkara zapota* and have appreciable anti diabetic activity.

CONCLUSIONS

The anti-diabetic properties of plants can be evaluated in vitro by several methods such as study of glucose uptake, effect on glycosylation of the haemoglobin and inhibition of alpha glucosidase and alpha amylase enzymes. The mechanism of glucose transport across they east cell membrane has been gaining significant importance as an *in vitro* screening method for evaluating the hypoglycaemic effects of various medicinal plants. The above conducted in vitro studies depict an appreciable increase in the glucose uptake by the yeast cells in combination with the plant extracts. It was observed that the plant extracts inhibited glycosylation of hemoglobin and thereby helps in the inhibition of the formation of glycated end products. We can therefore conclude from this study that the presence of the phytochemicals in these plants might be the reason for these inhibitions and that the plants may essentially contain herbal bioactive compounds which require further structural elucidation and characterization methodologies to identify the bioactive constituents. Further *ex vivo* and *in vivo* investigations should be done for confirming the anti diabetic activity of these plants. The plant extracts understudy can serve as therapeutic agents and can be used as potential sources of novel bioactive compounds for treating *Diabetes mellitus* type 2.

REFERENCES

1. Gershell L. (2005). Type 2 diabetes market. *Nat Rev Drug Discov.* 4, 367-68.
2. Tanko Y, Mohammed A, Musa K.Y and Eze E.D. (2012). Evaluation of Effect of Ethanolic Leaf Extract of Mucuna pruriens on Blood Glucose Levels in Alloxan-Induced Diabetic Wistar Rats. *Asian Journal of Medical Sciences,* 4(1), 23-28.
3. Bhalodi M, Shukla S, Saluja A.K. (2008). In Vitro antioxidant activity of the flowers of Ipomoea aquatic forsk. *Pharmacogn. Mag*., 4, 220-226.

4. Bhandari MR, Anurakkun NJ, Hong G, Kawabata J. (2008). Alpha glucosidase and alpha amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (Bergenia ciliata , Haw.). *Food Chem*, 106, 247-52.
5. Jung M, Park M, Chul HL, Kang Y, Seok-Kang E, Ki-Kim S. (2006). Antidiabetic agents from medicinal plants. *Curr. Med. Chem.*, 13, 1-16.
6. Ren J, Gintant GA, Miller RE, Davidoff AJ. (1997). High extracellular glucose impairs cardiac E-C coupling in a glycosylationdependent manner. *Am J Physiol*. 273, H2876-83.
7. Amakura Y, Umino Y, Tsuji S, Ito H, Hatano T, Yoshida T, Tonogai Y. (2002). Constituents and their antioxidative effects in Eucalyptus leaf extract used as natural food additive. *Food Chem*. 77, 47–56.
8. VP Cirillo. (1963). Sugar transport in a psychrophilic yeast. *J Bacteriol*. 84, 485–491
9. Adisa R. A, Oke J, Olomu S. A, Olorunsogo O. (2004). Inhibition of human haemoglobin glycosylation by flavonoid containing leaf extract of Cnestis ferruginea. *Journal of the Cameroon Academy of Sciences* 4, 351-359.
10. Maier A, Asano T, Volker A, Boles E, Fuhrmann G F. (2002). "Characterisation of glucose transport in Saccharomyces cerevisiae with plasma membrane vesicles (counter transport) and intact cells (initial uptake) with single Hxt1, Hxt2, Hxt3, Hxt4, Hxt6, Hxt7 or Gal2 transporters". *FEMS Yeast Research* 2(4), 539–550.