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Evaluation of *in vitro* anti diabetic activity of two mangrove plant extracts: *Heritiera fomes* and *Sonneratia apetala*

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Abstract
The main objective of this work was to evaluate the *in vitro* anti diabetic activity of methanolic leaf extracts of two mangrove plants: *Heritiera fomes* and *Sonneratia apetala*. The selected leaf extracts were studied for their effect on inhibition of alpha amylase activity, glucose transport across yeast cells and glucose adsorption capacity. It was found that leaf extract of *Heritiera fomes* (HF) inhibited α-amylase more effectively than leaf extract of *Sonneratia apetala* (SA). HF leaf extract caused greater uptake of glucose by yeast cells than SA leaf extract at different concentrations. Glucose adsorption capacity of both leaf extracts was increased proportionately in a dose dependent manner. This investigation indicated that the selected plants possessed considerable *in vitro* anti diabetic activity. Found effects of this study need to be confirmed using *in vivo* models for its effective and safe therapeutic uses.

Keywords: *Heritiera fomes*, *Sonneratia apetala*, α-Amylase, yeast cells, *in vitro*

1. Introduction
Plants have served mankind to alleviate chronic diseases from the very beginning of civilization. They are reservoirs of numerous medicinal components. In the past large varieties of synthetic drugs were commercially produced by pharmaceutical industries [1] for the treatment of different ailments. With the continuous use of synthetic drug severe side effects and resistance of microbes have started to develop. Besides large populations cannot afford to get benefit from these drugs due to their high expense. During the last decades scientists have started to 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 for cancer, liver diseases and arthritis where allopathy finds no complete cure. The bioactive compounds of medicinal plants are used as antidiabetic, chemotherapeutic, anti inflammatory, anti arthritic agents where modern medicines do not provide satisfactory cure [2]. Diabetes mellitus is a chronic endocrinological disorder affecting the metabolism of carbohydrates, proteins, fat, electrolytes and water. It includes a group of metabolic diseases which are characterized by hyperglycemia. Currently, there is growing interest in herbal remedies of diabetes due to the side effects of oral hypoglycemic agents [3]. So the traditional herbal medicines from plants are used in the management of diabetes mellitus in recent years. Bhandari *et al.,* (2008) [4] estimated that more than thousand plant species are being used as folk medicine for diabetes. Antidiabetic action of these plants are mainly due to their chemical composition. Herbal products or plant products are rich in flavonoids, phenolic compounds, coumarins, terpenoids and other constituents which provide antihyperglycemic effects [5]. Several species of herbal drugs with potential antidiabetic activity have been described in the scientific literature again and again. Herbal drugs possess good effectiveness, fewer side effects in clinical experience and they are relatively low in costs [6]. Medicinal and natural herbal plant products are traditionally used from long time all over the world for the treatment of diabetes mellitus. The present work was to evaluate the *in vitro* anti diabetic activity of methanolic leaf extracts of *Heritiera fomes* and *Sonneratia apetala* by studying their effects on α- amylase activity, glucose uptake in yeast cells and estimation of their glucose adsorption capacity.

2. Materials and Methods
2.1 Collection and preparation of leaf extract
Fresh leaves of *Heretiera fomes* (Sundri) and *Sonneratia apetala* (Kaora) were collected from

Mangrove forest Sundarban (Karamjol area) which grow prominently throughout that area.

Fig 1: Leaves of *Heritiera fomes* (left) and *Sonneratia apetala* (right)

The fresh leaves were washed separately and carefully with distilled water to remove any extraneous materials. The fresh leaves were air dried under shade for 7 days then dried in the oven at 65 ˚C. And then the dried leaves were pulverized into coarse powder. About 1 kg of the each powder was extracted with 2.5 L of methanol for 48 hours using Soxhlet apparatus. Then the efficient and gentle removal of solvents from samples was done by Rotary Evaporator and then the extract left behind was stored at 4 °C in a refrigerator. Methanol is suitable for extraction than Ethanol, because most of the polar or mid polar compounds get dissolved in methanol as compare to ethanol and it has low boiling point and high vapor pressure. Hence it can be useful to separate the extracted compounds by easy drying.

2.2 α-amylase Inhibition Assay

α- Amylase activity was carried out using the starch-iodine method. The reaction mixture contained 10 µl of α-amylase solution (10 mg/ml), phosphate buffer (0.02 M, pH 7.0) with 0.006 M NaCl (0.4 ml) and 1% starch solution (0.1 ml). After incubation at 37°C for 10 min, the starch solution was added, and the mixture was re-incubated for 1 h. Hereafter, 0.1 ml of 1% iodine solution was added, and after the adding of 5 ml distilled water the absorbance was taken at 565 nm. Sample, substrate and αamylase blank determinations were undertaken under the same conditions. The above experiment was conducted using different starch solutions [7]

2.3 Estimation of Glucose uptake in Yeast cells in presence of leaf extracts

The commercial baker's yeast was added to distilled water. This suspension was subjected to repeated centrifugation (3,000×g, 5 min) until clear supernatant fluids were obtained. Then a 10% (v/v) of the suspension was prepared in distilled water. Various concentrations of plant extracts (25- 200 µg/mL) were added to 1mL of glucose solution (10 mM) and incubated together for 10 min at 37 °C. 100µL of yeast suspension was added to the previous mixture and vortexed. After incubation at 37 °C for 60 min, the tubes were centrifuged (2,500 × g, 5 min). The amount of glucose was then estimated in the supernatant [8]. Metronidazole was used as standard drug. The percentage increase in glucose uptake by yeast cells was calculated as:

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

Where, Abs. of control= absorbance of the control reaction (containing all reagents except the test sample); Abs. of sample= absorbance of the test sample.

2.4 Determination of glucose adsorption capacity

Glucose adsorption capacity of the samples was determined by the method of Ou et al [9]. Briefly, the samples of plant extracts (1%) were added to 25 mL of glucose solution of increasing concentration (5, 10, 20, 50 and 100 mmol/L). The mixture was stirred well, incubated in a shaker water bath at 37 °C for 6 h, centrifuged at 4 800 r/min for 20 min and the glucose content in the supernatant was determined. The concentration of bound glucose was calculated using the following formula:

$$\text{Glucose bound} = \frac{G1 - G6}{\text{Weight of the sample}} \times \text{Volume of solution}$$

Where, G1= glucose concentration of original solution; G6= glucose concentration after 6 h.

3. Results and Discussion

3.1 α- amylase Inhibition Assay

The intestinal digestive enzymes α- amylase plays a vital role in carbohydrate digestion. One antidiabetic therapeutic approach is to reduce the post prandial glucose level in blood by the inhibition of α-amylase enzyme. These can be an important strategy in management of blood glucose [11].

Figure 2 Illustrates that leaf extract of *Heritiera fomes* (HF) has more potency to inhibit α-amylase than leaf extract of *Sonneratia apetala* (SA). The sequence of higher inhibition activity are as follows: Acarbose (Std.)> HF> SA. Though both of the leaf extracts showed less inhibitory action than the standard drug Acarbose at 0.5 mg/ml, 1 mg/ml and 5 mg/ml concentrations, at 2 mg/ml concentration they inhibited on α-amylase enzyme as efficiently as the standard drug (near to 100% inhibition). This figure also signifies that, increasing leaf concentration does not guarantee greater inhibitory action as enzyme inhibition decreased at 5 mg/ml concentration In spite of increasing the concentration at 5 mg/ml, their inhibition activity fall markedly. One explanation behind this may be the saturation of α-amylase enzyme with the substrate from leaf extract causes reduction of inhibitory action of the enzyme at the high level of the enzyme.

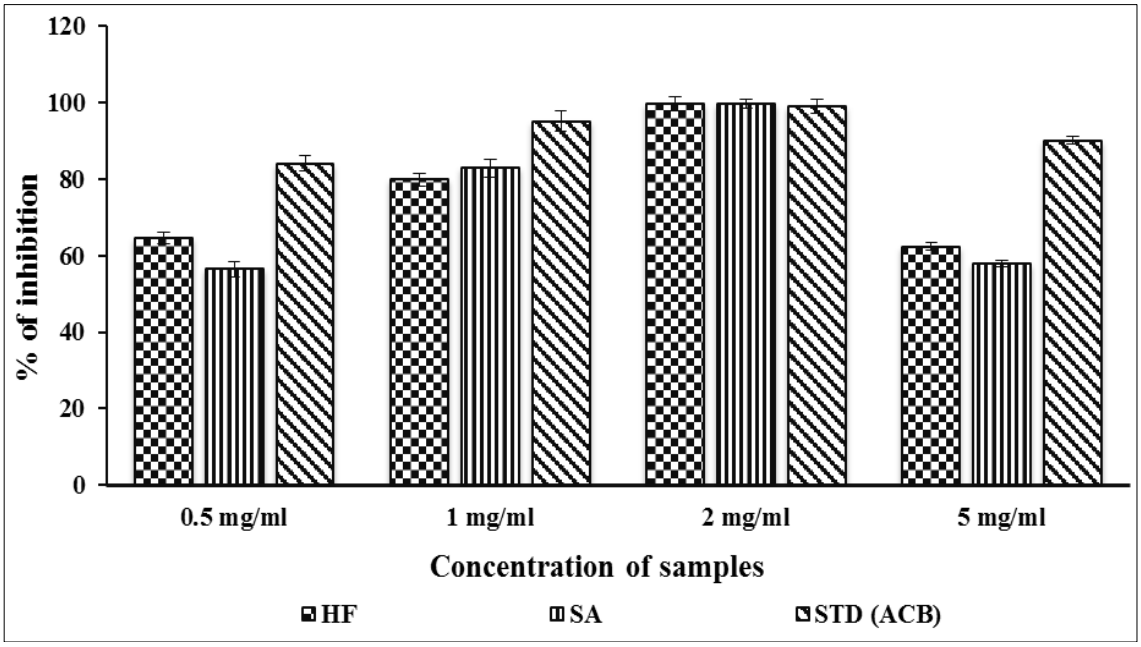

Fig 2: The effect of different extracts on α-amylase inhibition property.
HF= *Heritiera fomes*, SA= *Sonneratia apetala*, STD (ACB) = Standard Acarbose (Values are shown as mean± SEM of triplicate determinations)

3.2 Assay of Glucose Uptake in Yeast cells

Regulation of blood glucose level of the diabetic patient can prevent the various health complications. The long term maintenance of plasma glucose concentration under a variety of dietary conditions is one of the most important and closely regulated processes in mammalian species [11]. The *in vitro* assays of the present study indicated that the two plants: *Heritiera fomes* and *Sonneratia apetala* possess good anti diabetic activity. Yeast (*Saccharomyces cerevisiae*) transports glucose 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. Figure 3 depicts the % increase in glucose uptake by the yeast cell at different glucose concentrations i.e. 25mM, 50mM, 100mM and 200 mM respectively. This figure shows that HF leaf extract caused greater uptake of glucose by yeast cells than SA leaf extract at 25 µg/ml, 50 µg/ml and 100 µg/ml concentrations. At 200 µg/ml, glucose uptake in yeast cells was higher in the presence of SA. Even HF showed better result than the standard drug at the concentration of 50 µg/ml. The results obtained at 100 µg/ml was the most satisfactory as the uptake level was very good within a very narrow limit of standard deviation. Increasing leaf concentration does not necessarily increase cellular glucose uptake as observed at 200 µg/ml concentration.

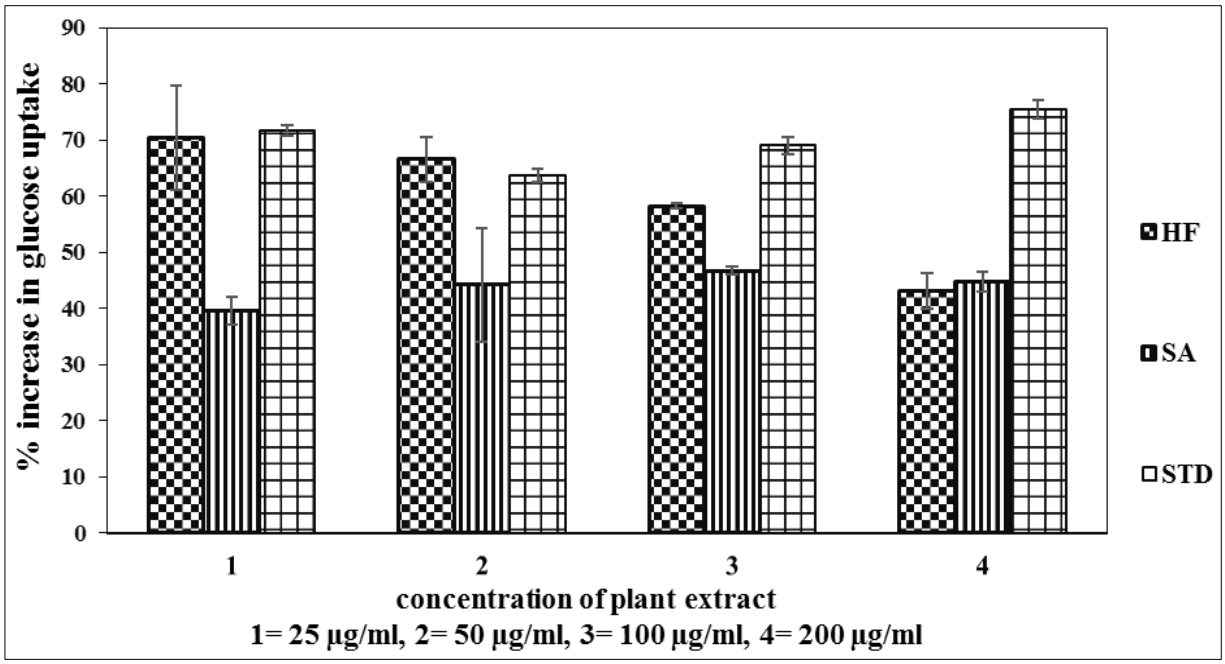

Fig 3: Estimation of Glucose uptake in yeast cell in presence of selected plant extracts.
HF= *Heritiera fomes*, SA= *Sonneratia apetala*, STD= Standard Metronidazole (Values are shown as mean± SEM of triplicate determinations)

After the treatment of the yeast cells with the methanolic leaf extracts, the glucose uptake was found to increase in a dose dependent manner. The figure 4 depicts the % increase in glucose uptake by the yeast cell at 10 mM glucose concentration. The methanolic extracts of *Heritiera fomes* leaf exhibited significantly higher activity than *Sonneratia apetala* leaf extracts at selected glucose concentration. Results also indicated that *Heritiera fomes* had greater efficiency in increasing the glucose uptake in yeast cells as compared to standard drug Metronidazole at 50 µg/ml concentration.

3.3 Assay of glucose adsorption capacity

Glucose adsorption capacity of the selected plant extracts is shown in Figure 4. The adsorption capacities of the samples were found to be directly proportional to the molar concentration of glucose and higher amounts of glucose was bound with increased leaf concentration. No significant ($P \leq 0.05$) differences were observed between the adsorption capacities of *Heritiera fomes* and *Sonneratia apetala*.

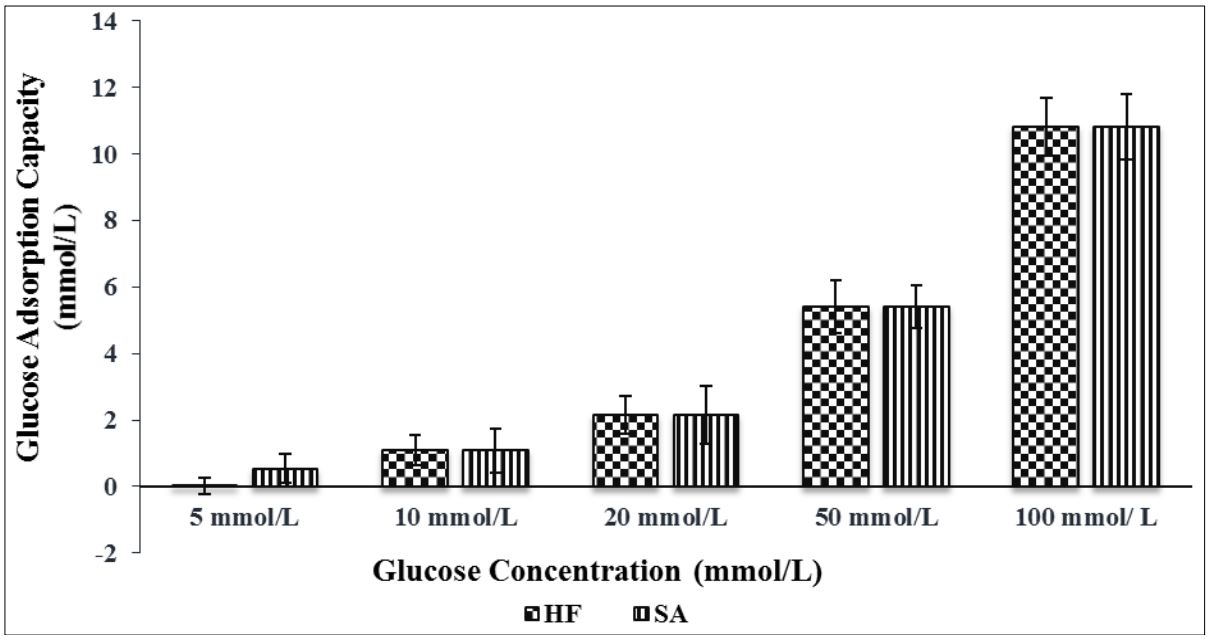

Fig 4: Comparison of Glucose Adsorption Capacity between two plant extracts.

HF= *Heritiera fomes*, SA= *Sonneratia apetala* (Values are shown as mean± SEM of triplicate determinations)

As shown in the above figure, glucose adsorption capacity of leaf extracts increased proportionately in a dose dependent manner. The results also revealed that the plant extracts under study could bind glucose even at lower concentrations of glucose (20 mmol/L) thereby reducing the amount of glucose available for transport across the intestinal lumen, consequently blunting the postprandial hyperglycemia. Both of leaf extracts adsorbed glucose of all concentrations to the similar extent and maximum adsorption was found at 100 mmol/L.

4. Conclusion

The antidiabetic properties of plants can be evaluated *in vitro* by several methods such as study of glucose uptake, inhibition of alpha glucosidase and alpha amylase enzymes and assay of glucose adsorption capacity. The mechanism of glucose transport across the yeast cell membrane has been gaining significant importance as an *in vitro* screening method for evaluating the hypoglycemic effects of various medicinal plants [12]. The above conducted *in vitro* studies depicted an appreciable increase in the glucose uptake by the yeast cells in presence of the plant extracts. It was observed that the leaf extracts inhibited α-amylase enzyme to different extent. The extract also showed a dose dependent increase in glucose adsorption capacity. 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. These compounds require further structural elucidation and characterization. Further ex vivo and *in vivo* investigations should be undergone for confirmation of the antidiabetic 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 type 2 Diabetes mellitus.

5. Conflict of Interests

The authors have no conflicts of interests.

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