IN VITRO EVALUATION OF ANTIDIABETIC ACTIVITY OF THE METHANOL LEAF EXTRACT OF *STERCULIA FOETIDA* LINN.

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ABSTRACT:

Diabetes Mellitus is a chronic metabolic disorder with altered carbohydrate, lipid and protein metabolism due to insufficient amount of insulin secretion by the organ pancreasin the human body. The synthesis of currently available anti-diabetic drugs like metformin, sulfonylureas etc., are mainly by the combination of artificial chemical molecules which lead to the various adverse effects. In recent years, the scientists have turned their attention towards the medicinal plants which bears the rich source of metabolites which offer specific physiological function in the human body without any adverse effect. Through literature, it was confirmed that in the olden days the bark, leaf, fruits, seeds of the plant *Sterculia foetida* were used as the medicine without the knowledge the actual bioactive molecules present in the parts of the plant. Among the various parts of the plant *Sterculia foetida*, the leaves bears various pharmacological activity and the current study elicited that the methanol leaf extract of *Sterculia foetida* were subjected to *invitro* antidiabetic activity by α-amylase enzymes and α-glucosidase enzymes against the standard acarbose. The result of the study provided us the strong proof that the glucose level was gradual decrease in the methanol leaf extract by inhibiting the function of α-amylase and α-glucosidase enzymes when compared with the standard acarbose. The outcome of the current study will pay the way for next step of research in methanol leaf extract of *Sterculia foetida* in discovering the new diabetic lead molecule for drug designing.

Keywords: *Sterculia foetida*, Methanol leaf extract, anti-diabetic activity, α-amylase enzyme, α-glucosidase enzyme.

1. INTRODUCTION:

One of the important metabolic disorders was Diabetes mellitus which affect all age groups due to the abnormal high blood sugar levels [1]. On latest diabetes survey revealed that In India more than 50 million people were suffering from type-2 diabetes on comparing with other countries. The World Health Organization estimated that 80 % of diabetes deaths occurred only in the low, middle level countries and also predicted that diabetes death will double between the years of 2016 to 2030 [2 - 4]. They also suggested that for India, diabetic people will increase up to 87 million people before 2030 [5]. So there was an urgent need for the development of new diabetic drug molecules without any side effects.

India was a founder and has a strong base towards Ayurvedic treatment. In Ayurveda, medicinal plants play a crucial role in preventing and curing many of human diseases. Indian food styles are rich in many plant phytochemicals ingredients which were used till now without knowing their pharmacological effect in the humans [6]. From the 21st century onwards, Indian traditional plants were mainly analysed by scientist to explore its phytochemicals with pharmacological action induced in the body there by preventing disease to enter inside the body [7].

According to WHO 2004, the use of traditional medicine as a complementary and alternative medicine were continue to expand quickly across the world for its phytonutrients by most of the people for treatment of various disorders in different national medical setting. They also reported that over past decade acceptance of traditional plant as natural therapies have been increased in both developing and developed countries [8]. Approximately about 80% of the world populations directly or indirectly rely on traditional medicine for their primary aid. They finally reported that in the next few decades, uses of traditional medicine may become a new era of medical science for the management and treatment for many human diseases [9]. The medicinal plants supplied the natural nutrients to humans and played the major role in preventing disease by scavenging the free radicals produced in the body [10].

The identification and isolation of bioactive molecules have paved way for the synthesis of novel natural drugs with multi targeted attitude towards treatment of the diseases. Hence above described pharmacological management of Type 2 diabetes mellitus leads some adverse effect on patients which pay a way for the scientist to discover the alternative medication without side effects [11]. Nowadays scientist and researchers started working on natural resources which inturn resulted in the identification of natural small molecules with fewer side effects [12 - 14]

In the current study on analyzing the various mallow families for its pharmacological activity, it was identified that the plant *sterculia foetida* was rich in various medicinal properties like anticonvulsant effect, antioxidant activity, anti-inflammatory activity, antidermatophytic activity, antifeedant activity, antiobesity activity, antifertility activity and mitogenic activity [15, 16]. The enzyme α-amylase and α-glucosidase serves as the major digestive enzymes and also helps in the intestinal absorption. The enzymes play an important role in the digestion of carbohydrate by breaking the long chain of polysaccharides, disaccharide into monosaccharides. They also suggested that the inhibitory action of these enzymes may reduce the absorbtion of blood glucose in the body [17]. The natural bioactive small molecules involves in delaying the absorption of glucose by inhibiting the pancreatic α-amylase and α-glucosidase enzymes, which was consider to be a carbohydrate hydrolysing and catalyzing enzymes. They also concluded that the medicinal plant bears various bioactive molecules in the all parts of the plant, the bioactive molecules has potential to exhibit high inhibitory activity on both the enzymes α-amylase and α-glucosidase [18].

The current study was designed in order to investigate the efficacy of antidiabetic property of methanol leaf of *Sterculia foetida* linn. For evaluating the antidiabetic effect of methanol leaf *Sterculia foetida* linn was determined by the regulatory effect on α-amylase enzyme and α-glucosidase enzyme which was consider to be a major carbohydrate metabolizing enzymes under *in vitro* conditions.

2. MATERIALS AND METHOD

2.1 Preparation of Plant leaves and its extracts:

The matured and freshleaves of *Sterculia foetida* were collected in the month of April, 2014 near Tambaram, Tamilnadu. The identification and authentication was done by Dr. P.Jayaraman, Director of Plant Anatomy Research Centre, Tambaram Further leaves were collected washed and dried for two weeks and then made into coarse powder.The powdered leaf (100 grams) was subjected to successive extraction with methanol solvents using soxhlet apparatus by continuous percolation process. Using rotary vacuum evaporator the leaf extract were collected and dried.

2.2 Screening of antidiabetic properties for the methanol leaf extract of *Sterculia foetida.*

To assess the antidiabetic properties of methanol leaf extract of *Sterculia foetida* was performed for two assays they are as follows:

- Alpha-amylase inhibitory activity.
- Alpha- glucosidase inhibitory activity.

2.2.1 Screening of *in vitro* alpha-amylase inhibitory activity for methanol leaf extract of *Sterculia foetida:*

Chemical:

- 0.1% (W/V) of Starch Solution.
- 0.1 g of Potato Starch.
- 16 mm of Sodium Acetate.
- α -Amylase Enzyme.
- Distilled Water.

Colorimetric Reagent:

- Sodium Potassium Tartarate.
- 3, 5 di nitro salicylic acid (96 mm).

Standard Drug – Acarbose.

Procedure:

The 0.1% (w/v) of starch solution was mixed with 0.1 g of potato starch in 100 ml of 16 mm of sodium acetate buffer. Then 27.5 µg of alpha-amylase enzyme was dissolved in 100 ml of distilled water. The solution of sodium potassium tartarate and 3, 5 di nitro salicylic acid (96 mm) was mixed well and were taken as the colorimetric reagent. The standard drug acarbose, was one of well-known anti-diabetic drug were taken as a control. The methanol leaf extract and standard were taken in the concentration of 200 - 1000 µg with the interval of 200 µg The methanol extract and standard were added to the above prepared starch solution and allowed to react with the enzyme solution of alpha- amylase under alkaline conditions at 25ºC. For every 3 minutes the reaction was observed for the generation of sugar molecules (maltose). The maltose was generated in the solution by the reduction of 3, 5 dinitro salicylic acid to 3- amino-5- nitro salicylic acid [19].

Detection of inhibition:

Finally the reaction was measured at 540 nm using colorimetry. The Inhibition of α-amylase inhibitory activity was calculated by using following formula: % Inhibition = $\frac{\text{(Absorbance of control – Absorbance of leaf extract)}}{\text{Absorbance of control}}$ X 100

2.2.2 Screening of *in vitro* alpha - glucosidase inhibitory activity for methanol leaf extract of *Sterculia foetida.*

Chemical:

- 2 % (W/V) of Starch Solution.
- 0.2 M Tris buffer pH 8.0.
- Alpha-Glucosidase enzyme (1U/ml).
- 6N Hydrochloric acid (HCl).

Standard Drug: Acarbose.

Procedure:

The 1 ml of (2 % w/v) of starch solution was mixed well with 0.2 M Tris buffer pH 8.0.To the above solution the various concentrationof leaf extracts were added. Incubate the solution for 5 min at 37°C for determining the inhibitory activity. The 1 ml of alpha-glucosidase enzyme (1U/ml) was added 40 min at 35°C to initiate the reaction further. Finally by adding 2 ml of 6N HCl, the reaction was terminated [20].

Detection of inhibition:

Finally the reaction was measured at 540 nm using colorimetry. The Inhibition of α-glucosidase inhibitory activity was calculated by using following formula: % Inhibition = $\frac{\text{(Absorbance of control – Absorbance of leaf extracts)}}{\text{Absorbance of control}}$ X 100

3. RESULTS AND DISCUSSION:

Screening of antidiabetic properties for the methanol leaf extract of *Sterculia foetida.*

Further screening of antidiabetic activity of the methanol leaf extract of *Sterculia foetida* were evaluated by *in vitro* models. The screening tests aids in tracing the presences of antidiabetic activity in the methanol leaf extract when compared with the standard acarbose.

3.1*In vitro* α-amylase inhibitory activity for leaf extract of *Sterculia foetida*.

The enzyme α-amylase inhibitory activity of standard acarbose and obtained methanol leaf extract of *Sterculia foetida*. The enzyme α-amylase helps in the hydrolyses of alpha bonds of polysaccharides i.e. starch and glycogen into simple sugars of glucose and maltose. The α-amylase inhibitors block the function of enzyme by binding to alpha bonds present in the polysaccharide. It was also observed that as the concentration of methanol leaf extract and standard increases (Table - 1); the percentage of α-amylase inhibitory activity also increases due to increased dosage of compounds present in the leaf extract and standard.

Table-1: Assessment of α-amylase inhibitory activity obtained from methanol leaf extract of *Sterculia foetida* and standard Acarbose:

S.No.	Conc of sample (µg/ml)	Methanol methanol leaf extract	Standard Acarbose
1.	200	27.8 %	33.7%
2.	400	45.9%	49.2%
3.	600	57.5%	59.4%
4.	800	66.2%	67.8%
5.	1000	71.3%	73.9%

The standard acarbose exhibited the maximum control of the enzyme α-amylase than methanol leaf extract of *Sterculia foetida* at a concentration 200 µg/ml. Likewise in the concentration of 400 µg/ml standard acarbose showed slight control over the enzyme α-amylase than methanol extract. Whereas in the other three increased concentration of 600 µg/ml, 800 µg/ml, 1000 µg/ml there was minute difference of inhibitory activity between both standard and methanol leaf extract of *Sterculia foetida*.

Further the result showed the dose dependent inhibitory action of standard and methanol leaf extract of *Sterculia foetida*. The result of α-amylase inhibitory activity depicts that the methanol leaf extract of *Sterculia foetida* exhibited thegood inhibitory activity of enzyme α-amylase by comparing with the standard acarbose. It also clearly revealed that if concentrations of methanol leaf extract increase gradually, the percentage of inhibitory activity of the enzyme also increased gradually. The result confirmed that the phytochemicals present in the methanol leaf extract was responsible for the inhibitory activity of the enzyme α-amylase.

3.2 Evaluation of *in vitro* α- glucosidase inhibitory activity for leaf extract of *Sterculia foetida.*

The enzyme α-glucosidase inhibitory activity of standard acarbose and methanol leaf extract of *Sterculia foetida* Table - 2. The enzyme α - glucosidase catalyses the cleavage of disaccharides to form simple sugar. The α - glucosidase inhibitors can delay the uptake of dietary sugars and also suppress the post-prandial hyperglycemia. It was also observed that as the concentration of methanol leaf extract and standard increases; the percentage of α-glucosidase inhibitory activity also increases due to increased dosage of compounds in the leaf extract and standard.

The standard Acarbose exhibited the maximum control of the enzyme α-glucosidase than methanol leaf extract of *Sterculia foetida* at a concentration of 200 µg/ml. Likewise in the concentration of 400 µg/ml standard acarbose showed slight control over the enzyme α-glucosidase than methanol extract. Whereas in the concentration of 600 µg/ml and 800 µg/ml, the methanol extract showed a slight variation in the inhibitory activity of enzyme when compared with the standard acarbose. In the maximum concentration of 1000 µg/ml, there was minute difference in the inhibitory activity between standard and methanol leaf extract of *Sterculia foetida*.

Table-2: Screening of α- glucosidase inhibitory activity from the methanol leaf extract of *Sterculia foetida* against standard Acarbose.

S.No.	Conc. of sample (µg/ml)	Methanol leaf extract	Standard Acarbose
1.	200	31.7%	40.7%
2.	400	42.6%	49.3%
3.	600	58.2%	63.4%
4.	800	66.5%	72.2%
5.	1000	89.4%	91.5%

Further the result showed the dose dependent inhibitory action on standard and methanol leaf extract of *Sterculia foetida*. The result of α-glucosidase inhibitory activitydepicts that the methanol leaf extract of *Sterculia foetida* exhibited the good inhibitory activity of enzyme α-glucosidase by comparing with the standard acarbose. It also clearly revealed that if concentrations of methanol leaf extract increase gradually, the percentage of inhibitory activity of the enzyme α-glucosidase also increased gradually.

The result of *in vitro* antidiabeticstudies, determined that the methanol leaf extract of *Sterculia foetida* exhibited the better antidiabetic activity on both the enzymes α-amylase and α-glucosidase. By comparing with the standard acarbose, methanol extract exhibit the similar inhibitory activity on both enzyme in higher concentrations (600 µg/ml, 800 µg/ml, 1000 µg/ml). The obtained result was further analysed and it

was clearly revealed that as the concentration increased, the inhibitory activity of methanol extract also increased in both enzymes α- amylase and α- glucosidase.

The previous preclinical evaluation of antidiabetic study [21] revealed that the methanol leaf extract of *Sterculia foetida* possess significant antidiabetic activity in the wistar albino rats and also restored the metabolic changes in the inalloxan-induced diabetic rats. They also concluded that the methanol extract is very effective in lowering the fasting blood glucose level of the diabetic wistar albino rats. The earlier study on inhibitory action of α-amylase and α-glucosidase of some plant extracts revealed that the bioactive molecules present in the plant extract may act as the strong α-amylase inhibitors and α-glucosidase inhibitors. They also suggested that the identification and isolation of bioactive molecules play a very vital role as lead molecules in the designing and development of antidiabetic drug [22]. The previous study on antidiabetic activity of ethanol and aqueous flower extracts of *Sterculia foetida* on streptozotocin induced diabetic rats exposed that the ethanol and aqueous flower extracts of *Sterculia foetida* showed the significant reduction in blood glucose levels of diabetic rats. They suggested that the component present in the flower of *Sterculia foetida* must be identified for the further investigation of diabetic activity [23].

From the above previous study it was clearly revealed that the bioactive molecules present in the plant *Sterculiafoetida*play a vital role as antidiabetic properties. The current study of the antidiabetic screening test revealed that the methanol leaf extract of *Sterculia foetida* exhibit the better antidiabetic activity. Further isolation and identification of the bioactive compounds were carried out to determine the exact bioactive molecule which is responsible for the antidiabetic properties.

4. CONCLUSION:

The plant *Sterculia foetida* were distributed throughout India and in traditional medicine the plant were used in wide range because of its cheap and easily accessible remedy for common man to cure the disease without any side effects. The result of *in vitro* studies on methanol leaf extract of *Sterculia foetida*provided us scientific evidences that the leaf extract exhibit the better antidiabetic property.The evaluation of antidiabetic property was carried out through the inhibition activity of methanol leaf extract towards the enzyme alpha amylase and alpha glucosidase. The study also explored that the methanol leaf extract exhibit the similar inhibitory action of standard acarbose. The phytoconstitutes responsible for antidiabetic activity are not currently predicted and in our future research the identification and isolation of major antidiabetic phytoconstitute present in the methanol leaf extract of *Sterculia foetida* will lead to the discovery of new anti-diabetic drug molecule.

5. CONFLICT OF INTEREST:

The authors declare they have no competing interests.

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