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Comparative Study of *Costus igneus* and Metformin in *In-Vitro* Inhibitory Activity of α-Amylase and α-Glucosidase Activity

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ABSTRACT

The objective of the present study was to compare the α-amylase and α-glucosidase inhibition activity of aqueous extracts of costus igneus with that of metformin in in-vitro studies. Different concentrations of the aqueous extract of the leaf from the plant costus igneus and the solution of drug metformin (100-500 µl) and standard drug (Acarbose) were taken and assessed for the antidibetic activity based on their α-amylase and α-glucosidase inhibition.

The alpha-amylase inhibition action of Costus igneus was 89% of that of metformin at a concentration of 500 µg. The alpha-glucosidase inhibition action of Costus igneus was 66.6% of that of metformin at a concentration of 500 µg. The study has shown good results and could be a potential topic for exploration of antidiabetic properties. The aqueous extract of the leaves of Costus igneus could serve as an alternative to the commonly used antidiabetic drug metformin. However further studies need to be performed to assess its safety profile.

Key words: Acarbose, Alpha-glucosidase, Metformin

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INTRODUCTION

Diabetes mellitus is one of the most commonly occurring endocrinological diseases in today's world. The incidence of diabetes in constantly increasing with the graph showing a steeply rising positive slope [1].

Type II diabetes mellitus was initially the disease of the late tricenarian or Quadragenarian but now due to the rapidly changing lifestyle people get affected earlier.

A sedentary lifestyle and consumption of foods rich in refined sugars has played a significant role in the pathogenesis of DM, besides high genetic predisposition [2].

Diabetes mellitus is characterised by a plethora of pathological changes which are responsible for the symptoms occurring in patients with DM.

The micro and macro vascular changes which occur as a consequence of the underlying raised levels of sugars are instrumental in long term complication seen in DM [3].

In a state of diabetes mellitus either the cells in the body become less sensitive to insulin and there by the plasma levels of insulin rises or the beta cells in the islets of Langerhans in the pancreas produce lesser amounts of insulin than normal. The treatment of diabetes mellitus is a multimodal approach. The treatment varies from delaying the absorption of sugars from the intestinal tract to increasing its excretion by the kidneys. The control of diabetes is still unsatisfactory in India with a random community level of around 8.9. Even though newer drugs with more specific targets are continuously being invented, the side effect profile also extends. To circumvent the problem, the age old, described plant products with action on the specific problem of type 2 DM is worthy. Alpha amylase and Alpha glucosidase are important enzymes that are responsible the breakdown of starch into oligosaccharides during the absorption from intestine. By accessing the Alpha amylase inhibition and Alpha glucosidase inhibition activity one can assess the antidiabetic action of the study substance. In this study we have compared the Alpha amylase inhibition and alpha glucosidase inhibition of *Costus igneus* and metformin to compare the Anti Diabetic profiles of the same [4].

***Costus igneus*:** Commonly known as insulin plants also has other name such as spiral flag, crepe ginger. This species is known to have an antidiabetic effect and there are many animal studies performed reflecting the antidiabetic property of the costus species on dexamethasone induced diabetes mellitus. Sequential

screening for phytochemicals of *Costus igneus* leaves revealed that it is rich in protein, iron, and antioxidant components such as ascorbic acid, α-tocopherol, β-carotene, terpinoids, steroids, and flavonoids. This plant was selected based on its ethnopharmacological use. The *Costus igneus* plant is recently grown in South India as the insulin plant and belongs to the family Costaceae. The extracts of the leaves of the plants have effect on reduction of blood glucose. There are studies are being carried out to determine the hypolipidemic, antioxidant, diuretic, anti-microial and anti-cancer property of *costus igneus* (Figure 1).

Figure 1: *Costus igneus* leaves.

Metformin: One of the most commonly used oral hypoglycemic agents. Dose of metformin ranges from 500-2500 mg/day [5].

It is presently the first line therapy for patients with Type 2 diabetes mellitus, according to the American diabetes Association of the study of diabetes guidelines [6].

Metformin works *via* various mechanism of actions such as inhibition of gluconeogenesis by activating AMP activated protein kinase, by decreasing intestinal glucose absorption, by improving peripheral glucose uptake, by lowering the fasting levels of insulin, by increasing the insulin sensitivity, all this without causing overt hypoglycemia. Metformin is in use for over 50 years and there are numerous studies concerning other indications and pleomorphic effects of the same [7].

MATERIALS AND METHODS

The study plant (*Costus igneus*) and control drug (metformin) were taken and subjected to evaluation of their antidiabetic effects [8].

Preparation of plant extract

Method: cold extraction

10 gm of fresh leaves sample was weighed and soaked in 100 ml of aqueous solution (Distilled Water). The extract was allowed to stand overnight and filtered using sterile filter paper. The filtrate was used for the antioxidant activity and antidibetic activity (Figures 2 and 3).

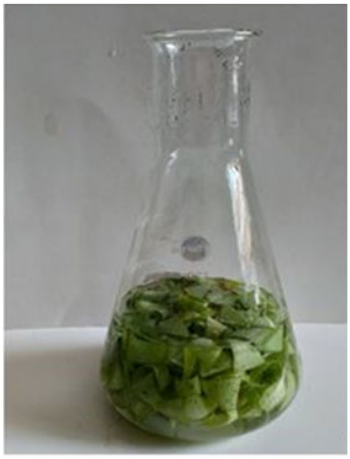

Figure 2: Cold extraction process of *costus igneus* leaves.

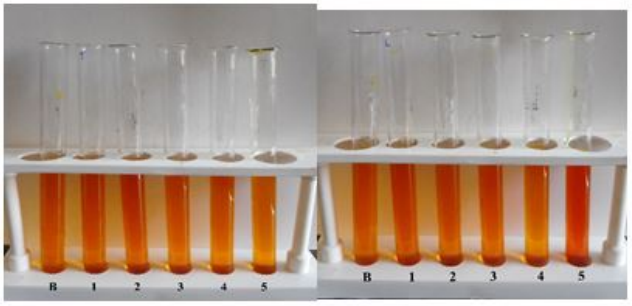

Figure 3: Filtration of cold extract.

Anti-diabetic activity

Inhibition of alpha amylase

Procedure: Different concentrations of samples (100-500 µl) and standard drug (Acarbose) were taken. Then 1 ml of α-amylase in 0.2 M sodium phosphate buffer (pH 6.9) was added to each tube and was incubated at 25°C for 30 min. Then 1 ml of 1% starch solution in 0.2 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 3 min. The reaction was stopped with 1 ml of 3, 5 dinitrosalicylic acid. 9 ml of distilled water was added to the reaction mixture. Absorbance was measured at 540 nm (Table 1).

Table 1: Inhibition of alpha amylase activity.

Sample Concentration (µg)	100	200	300	400	500
Tablet (Metformin)	0.31	0.35	0.41	0.46	0.5

% Inhibition	54.8	60	65.8	69.5	72
Leaf aqueous extract	0.25	0.29	0.32	0.36	0.39
% Inhibition	44	51.7	56.2	61.1	64.1

Inhibition of alpha glycosidase

Procedure: 100 µl of 0.1 U glucosidase was taken in different tubes. To this 100-500 µl of sample and standard (acarbose) of different concentrations were added (should not mix) and incubated at 25°C for 10 min. Then 50 µl of p-nitro phenyl alpha-D-glycosidase was added, vortexes and incubated at 25°C for 5 min. Add 800 µl of stop solution (0.1 M sodium carbonate) was added. Absorbance was measured at 405 nm (Table 2).

Table 2: Inhibition of alpha glycosidase activity.

Sample Concentration (µg)	100	200	300	400	500
Tablet	0.1	0.07	0.05	0.04	0.03
% inhibition	44.4	61.1	72.2	77.7	83.3
Leaf aqueous extract	0.16	0.14	0.11	0.09	0.08
% inhibition	11.1	22.2	38.8	50	55.5

Statistical analysis

The test plant extract and control drug solution were subjected to evolution of their anti-diabetic property by determining the alpha amylase and alpha-glycosidase inhibitory activity.

RESULTS

The results of their inhibitory activity were compared and the relative effect was calculated (Tables 3 and 4, Figures 4 and 5).

Table 3: Alpha-amylase activity.

Sample Concentration (µg)	100	200	300	400	500
Metformin % inhibition	54.8	60	65.8	69.5	72
Costus igneus **% inhibition**	44	51.7	56.2	61.1	64.1
Relative %	80.29	86.1	85.4	87.9	89

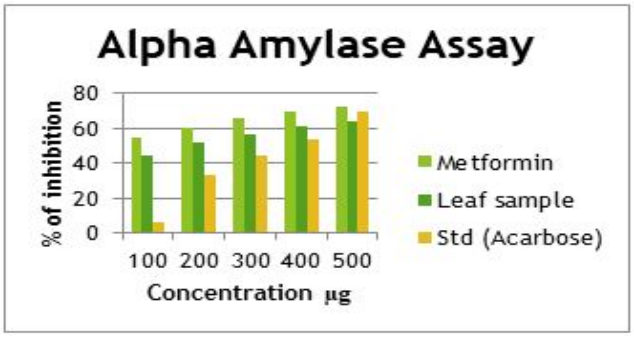

Figure 4: Alpha-amylase activity.

Table 4: Alpha glycosidase activity.

Sample Concentration (µg)	100	200	300	400	500
Metformin % inhibition	44.4	61.1	72.2	77.7	83.3
Costus igneus **% inhibition**	11.1	22.2	38.8	50	55.5
Relative %	25	36	53.7	64.3	66.6

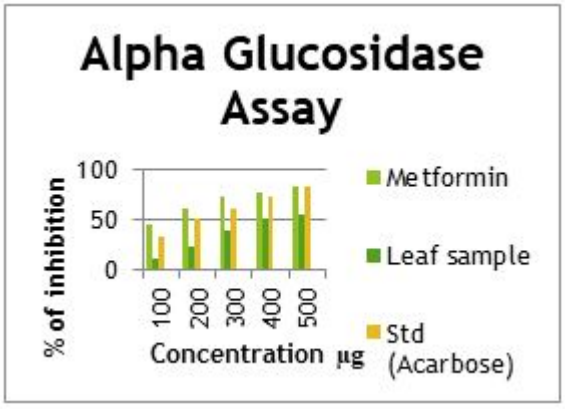

Figure 5: Alpha glucosidase activity.

DISCUSSION

Costus igneus showed upto 89% efficacy of metformin at a concentration of 500 µg of each in alpha-amylase inhibitory activity and 66.6% efficacy of metformin at a concentration of 500 µg of each in alpha-glucosidase inhibitory activity.

CONCLUSION

The antidiabetic effect of the aqueous extract *Costus igneus* leaf extract assessed by *in vitro* studies has shown upto 89% of that of metformin in the inhibition of alpha-amylase and 66.6% of that of metformin in the inhibition of alpha glucosidase.

The study has shown good results and could be a potential topic for exploration of antidiabetic properties. The aqueous extract of the leaves of *Costus igneus* could serve as an alternative to the commonly used antidiabetic drug metformin. However further studies need to be performed to assess its safety profile.

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