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Research Article

In-vitro Alpha-amylase and Alpha-glucosidase activity of Costus pictus D. Don and Costus speciosus (J. Koenig) Sm.

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ABSRACT:
Plants continue to be the source of all wealth. Living beings of the planet are blessed with a large number of plant with medical values, According to the World Health Organization (WHO), nearly 4 billion people, accounting roughly, 66.6 percent of the world's population use the medicinal plants as curative agents. Though the plants serve humans to maintain their well being the investigation of the medicinal values of herbs to combat various ailments became obligatory. Hence, there is an urgent need for constant screening of herbal drugs for better and effective utilization. Diabetes medically known as Diabetic Mellitus is a metabolic disorder marked by high blood sugar level over a prolonged period. It accounts for 42% of deaths in India. The enzyme α-amylase and α-glucosidase that catalyzes the hydrolysis of starch and sugar plays a vital role in the metabolic disorder, Diabetic Mellitus. *Costus pictus* and *Costus speciosus* exhibits antidiabetic activity. The present work is carried out to analyse the effect of inhibition of α-amylase and α-glucosidase enzymes by *Costus pictus* and *Costus speciosus*. *Costus pictus* leaf and rhizome extract showed inhibitory activity against both the enzymes. Similar such results were also observed in the leaf and rhizome of *Costus speciosus.*

Key words: Diabetic Mellitus, α-amylase, α-glucosidase, Costus pictus, Costus speciosus

I. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder that is characterized with increased blood glucose level (Hyperglycemia) and lasts for life. DM becomes to be a Cosmo political prevalence rooted to socio-economic consequences and 234% increase was witnessed during 1980 -2014. The global prevalence of diabetes* among adults over 18 years of age has risen from 4.7% in 1980 to 8.5% in 2014. Approximately, 285 million people (6.4% of the adult population) are affected by diabetes and it is expected to rise 370 million in 2030. In 2016, an estimated 1.6 million deaths were directly caused by diabetes. Another 2.2 million deaths were attributable to high blood glucose in 2012. Almost half of all deaths attributable to high blood glucose occur before the age, 70. WHO announced, diabetes as the seventh leading cause of death in 2016 (Roglic, 2016).

DM is caused by several improper metabolic alternations in insulin mechanism such as lower production by β pancreatic cells or its improper metabolic usage. These alternations directly or indirectly affect anabolism or catabolism of carbohydrates, lipids, and proteins. Its symptoms include polyuria (excessive urination, polyphagia (excessive appetite), polydipsia (excessive thirsty) and unreasonable weight loss (Mukhtar, 2019). DM causes a variety of damage to body organs including eyes, kidneys, nerves and blood vessels based on the disease prolongation. Inadequate disease control results in acute complications (*viz.,* hypoglycemia, ketoacidosis, non-ketonic hypersomolar coma) that stems for chronic complications that resulting in cardiovascular, retinopathy, neuropathy and micro vascular damage (Wang and Zaman, 2019).

1.1.1 Types of Diabetes

DM is classified into three main types namely, Type- I (Insulin dependent diabetes mellitus, IDDM), Type- II (Non - Insulin dependent, DDM) and gestational diabetes. Type1 arises due to the damage in pancreatic β cells that are responsible for insulin secretion. These damaged β cells secrete little or more insulin and resulting in poor sugar metabolism (Bhatt *et al*., 2019; Saunders and Powers, 2016). People with type1 diabetes must use insulin injections to control their blood glucose level. Type I is the most common form of diabetes. it is also known as juvenile diabetes. Type II DM occurs while the body mechanism doesn't obey to insulin properly. It usually affects the younger over the age of 40 and becomes a threat to youngest ones nowadays. The gestational diabetes occurs at the time of pregnancy since the placental hormones also blocks the functions of mother's insulin and eventually raise the blood glucose level (Lewis *et al*., 2018). Usually women older than 25 years, with excessive body weight and who have familial diabetic history are more prone to have gestational diabetes.

1.1.2 Causes of diabetes

The DM is caused by many unrelated factors such as family history of diabetes or inherited tendency; overweight (20% or more over than desired body weight). Prolong physical stress characterized by surgery or illness also causes the diabetes. Damage to pancreatic cells is a main reason for type 1 diabetes. Elevated blood pressure could increase the risk of diabetes. Abnormal cholesterol level and aging causes severe diabetic risks. Alcohol and smoking may increase the risk level to the alarming folds (Tran *et al*., 2017).

1.1.2 Symptoms of diabetes

Being a metabolic disorder, diabetic have different symptoms such as heavy thirst, increased hunger, dry mouth, nausea, vomiting, pain in the belly, frequent urination, fatigue and blurred vision etc., (Bhatt *et al*., 2019)

1.1.3 Diagnosis of diabetes

The preferred method of diagnosing diabetes is the fasting plasma glucose test (FPG). The FPG measures your blood glucose level after you have fasted for 10 to12 hrs. Normal fasting blood glucose is between 70 and 100 mg/dl for the people who do not have diabetes. Oral glucose tolerance test may aid in the diagnosis of diabetes. A1C test, measures your average percentage of glycated haemoglobin which tells about the blood glucose control for past 2-3 months (Kaur *et al*., 2018).

1.1.5 Hyperglycemia

DM is characterized by higher glucose level in blood (Hyperglycemia). It develops when the blood sugar surpasses the adequate level. The early signs and symptoms include frequent urination, increased thirst blurred vision and fatigue. At later, the people may feel fruity smelling breath, nausea, vomiting, and shortness of breath, dry mouth and abdominal pain. (Kaur *et al*., 2018)

1.1.6 Hypoglycemia

It is a condition characterized by an abnormally low level of blood sugar (glucose). It is commonly linked with diabetes. Hypoglycaemia is a symptom resulting from low blood glucose which are autonomic or neuroglycopenic. Autonomic symptoms include sweating, trembling, feelings of warmth, anxiety and nausea. Neuroglycopenic symptoms include feelings of dizziness, confusion, tiredness difficulty in speaking, headache and inability to concentrate (Kapoor *et al.*, 2017).

1.2 Pancreas

The pancreas is a gland organ that is located in the abdomen. It is a part of the digestive system and produce important enzymes and hormones that help to break down foods. The pancreas has an endocrine function because it releases juices directly into the blood stream and it has an exocrine function because it releases juices into the ducts. The gland also produces the hormone insulin and secretes it into the blood stream in order to regulate the body's glucose level or sugar level (Sonntag *et al*., 2018).

1.3 Diabetic therapy

The scientific advancements bring a variety of treatments against diabetics such as insulin therapy, pharmacotherapy, and diet therapy. These therapies mainly focus on controlling the diseases. Many drugs have glucose-lowering effects *in-vivo* through different mechanisms. Thesulfonylurea and meglitinides drugs usually stimulate insulin secretion. The biguanides and thiazolidinediones may increase the peripheralglucose absorption. Many drugs delay the carbohydrate absorption at intestines by inhibiting the alpha-glucosidase. Biguanides are reducing the hepatic gluconeogenesis. Drug resistance (reduction of efficiency), side effects and even toxicity are major side effects of antidiabetic drugs (Shi *et al*., 2019).

The plants contain carotenoids, flavonoids, terpenoids, alkaloids, glycosides and can often have anti-diabetic effects. These plant based compounds usually enhance the pancreatic cell activity for insulin secretion or reduce the intestinal glucose absorption. Siddha, Ayurveda and Unani are the members of Indian systems of Alternative medicines. They vary with formulae preparation for drug effects (Bilal *et al*., 2018).

India is considered as the Botanical Garden of the World due to rich medicinal flora present here. Medicinal drugs are primary health care taker in Indian population. These medicinal plants are sources of drugs with minimal side effect and low cost. The aim of the present work is to study the antidiabetic activity of the 4 samples.

2. OBJECTIVES OF THE RESEARCH

To evaluate the anti-diabetic activity of the leaf and rhizome of *Costus pictus* and *Costus speciosus* samples using α-amylase and α-glucosidase inhibition assays.

3. MATERIALS AND METHODS

Extraction of the Samples:

To extract the phytocompounds present in the sample, 2 grams of each sample was extracted by 100 ml of methanol at 60°C for 48 hours. Then the extract was filtered and concentrated. This will be used for the assays. Extractive value was also calculated.

Alpha-Glucosidase inhibition assay

Screening of the extracts for α-glucosidase was carried out according the method of Bhatia et al. (2019) with slight modifications. Varying concentrations of test samples were dissolved in DMSO and made up to 1ml. Solvent DMSO was used as control and Acarbose as positive control. 50 µl of α-glucosidase (0.01mg/ml) prepared in 0.1M phosphate buffer (pH 6.8) was added to all the tubes. The tubes were incubated for 5 minutes at 37°C. Then 20 µl of 5 mM pNPG also prepared in 0.01M phosphate buffer (pH 6.8) was added to all the tubes. Tubes were incubated at 37°C for 15 minutes. Finally 50 µl of 0.1M Na_2Co_3 was added to stop the enzymatic reaction. Absorbance was measured at 405nm. Inhibition concentration was calculated using the formula given below,

$$\text{Inhibition (\%)} = [(\text{Absorbance}_{\text{Blank}} - \text{Absorbance}_{\text{Sample}}) / \text{Absorbance}_{\text{Blank}}] \times 100$$

Alpha-amylase inhibition assay

To determine the α-amylase inhibition activity of extracts, method demonstrated by Alqahtani *et al*. (2020) with slight modifications. Varying concentrations of test samples were dissolved in DMSO and made up to 1ml. Solvent DMSO was used as control and Acarbose as positive control were also prepared. 50 µl of porcine pancreatic amylase (0.5mg/ml) prepared in 0.01M phosphate buffer (pH 6.8) was added to all the tubes. Tubes were Incubated 10 minutes at 37°C. Then 50 µl of 1% soluble starch also prepared in 0.01M phosphate buffer (pH 6.8) was added to all the tubes. Again the tubes were incubated at 37°C for 15 minutes. Finally 100 µl of DNS Reagent was added to stop the enzymatic reaction. Heat all the tubes in boiling water bath for 5 minutes. Cool the tubes at room temperature and measure the absorbance at 540nm. Inhibition concentration was calculated using obtained values.

$$\text{Inhibition (\%)} = [(\text{Absorbance}_{\text{Blank}} - \text{Absorbance}_{\text{Sample}}) / \text{Absorbance}_{\text{Blank}}] \times 100$$

RESULTS:

The results were shown in Tables 1-5 and Figures 1-12. The dose dependent antidiabetic activity was observed in the leaf and rhizome extracts of *Costus pictus* and *Costus speciosus*. The higher activity was observed in rhizome of *Costus pictus* and rhizome of *Costus speciosus* when compared to acarbose.

The dose dependent antidiabetic activity was observed in *Costus pictus* leaf extract. The higher activity was observed to inhibit α-amylase enzyme than α-glucosidase enzyme. Acarbose was used as standard. The extractive value of *Costus pictus* leaf extract was found to be 4.86% using methanol.

The dose dependent antidiabetic activity was observed with *Costus pictus* rhizome extract. Higher activity was observed to inhibit α-amylase enzyme than α-glucosidase enzyme. Acarbose was used as standard. The extractive value of *Costus pictus* rhizome extract was found to be 2.8% using methanol.

The dose dependent antidiabetic activity was observed in *Costus speciosus* leaf extract. The higher activity was observed to inhibit α-amylase enzyme than α-glucosidase enzyme. Acarbose was used as standard. The extractive value of *Costus speciosus* leaf extract was found to be 8.8% using methanol.

The dose dependent antidiabetic activity was observed in *Costus speciosus* rhizome extract. The higher activity was observed to inhibit both α-amylase and α-glucosidase enzymes. Acarbose was used as standard. The extractive value of *Costus speciosus* rhizome extract was found to be 2.64% using methanol.

Table.1. Extractive Values

S. No.	Plant extracts	Weight of extract	Extract %
1.	*Costus pictus* leaf extract	0.0973 gm	4.86

2.	*Costus pictus* rhizome extract	0.056 gm	2.8
3.	*Costus speciosus* leaf extract	0.176 gm	8.8
4.	*Costus speciosus* rhizome extract	0.0528 gm	2.64

Table.2. Anti diabetic activity of *Costus pictus* and *Costus speciosus* using α - Glucosidase inhibitiory assay

Concentration of plant extracts	*Costus pictus*		*Costus speciosus*		OD Values of Acarbose
	OD Values of leaf extract	OD Values of rhizome extract	OD Values of leaf extract	OD Values of rhizome extract	
50 µg/ml	0.961±0.006	0.472±0.004	0.688±0.007	0.41±0.005	0.312±0.003
100 µg/ml	0.923±0.005	0.38±0.009	0.654±0.010	0.396±0.003	0.285±0.007
150 µg/ml	0.691±0.003	0.274±0.003	0.48±0.008	0.222±0.003	0.259±0.004
200 µg/ml	0.607±0.011	0.258±0.002	0.45±0.007	0.196±0.008	0.223±0.009
300 µg/ml	0.472±0.008	0.231±0.007	0.343±0.005	0.151±0.004	0.156±0.010

Table.3. Percentage of α-Glucosidase inhibitory activity

Concentration µg/ml	Percentage of Inhibition (%)				
	Costus pictus		*Costus speciosus*		Acarbose
	Leaf extract	Rhizome extract	Leaf extract	Rhizome extract	
100 µg	0	5.41	0	17.84	9.30
200 µg	0	23.85	0	20.64	17.15
300 µg	0	45.09	3.81	55.51	24.71
400 µg	0	48.30	9.82	60.72	35.17
500 µg	5.41	53.71	31.26	69.74	54.65

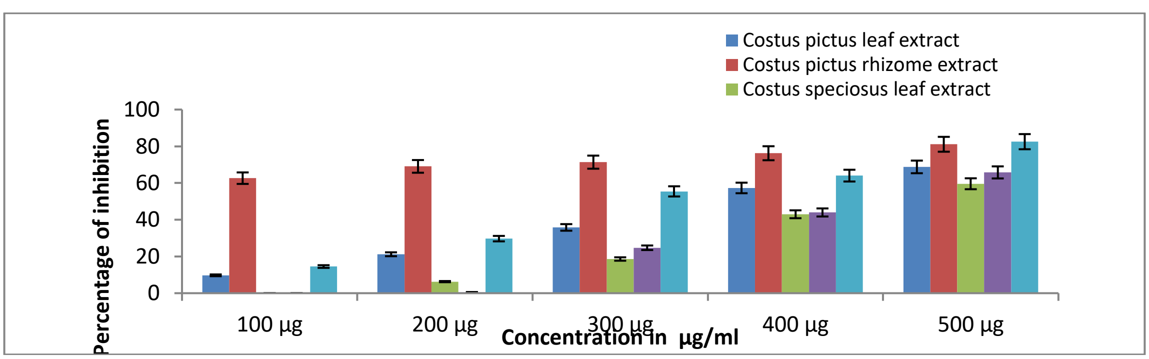


Fig. 1: α-Glucosidase inhibitory activity of *Costus pictus* and *Costus speciosus*

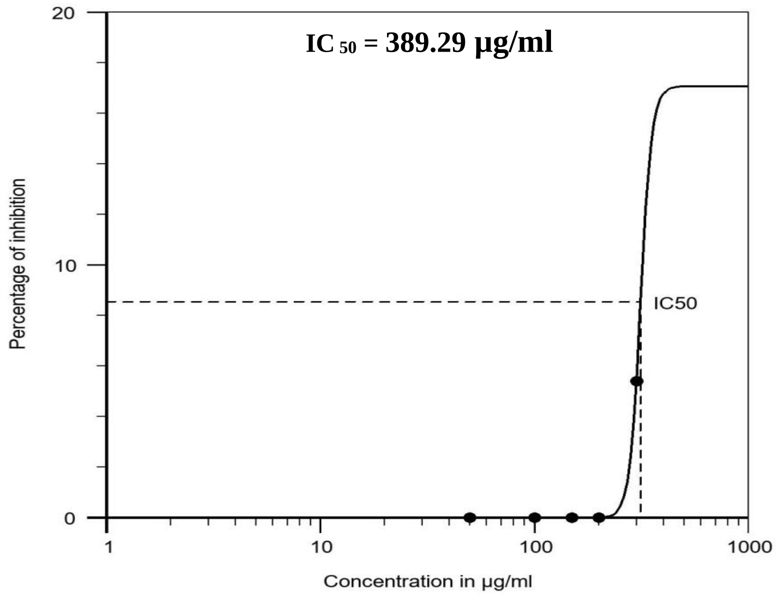


Fig.2. α - Glucosidase inhibitiory activity of leaf extract of *Costus pictus* at varying concentrations

IC $_{50}$ = 189.45 µg/ml

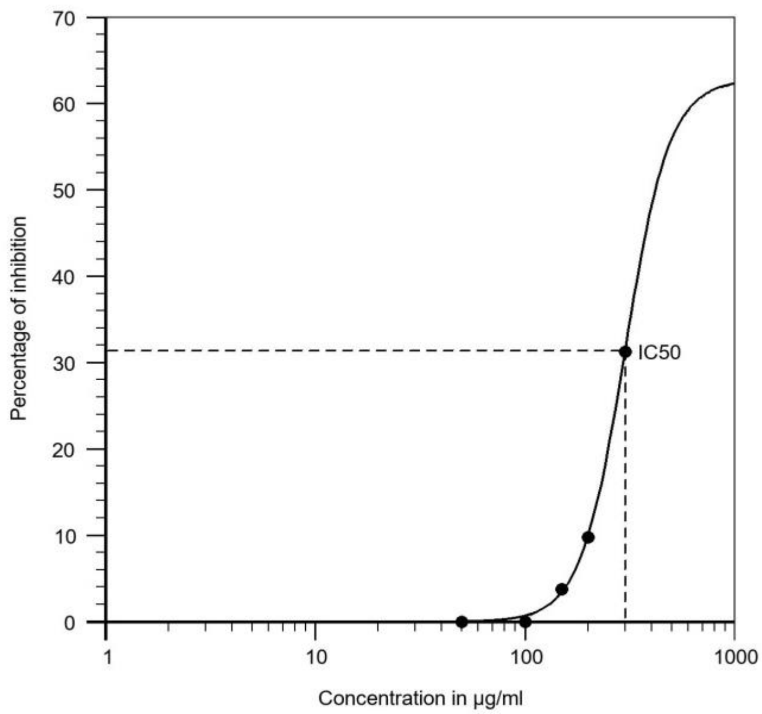


Fig.3. α - Glucosidase inhibitiory activity of rhizome extract of *Costus pictus* at varying concentrations

IC $_{50}$ = 348.4 µg/ml

Fig.4. α - Glucosidase inhibitiory activity of leaf extract of *Costus speciosus* at varying concentrations

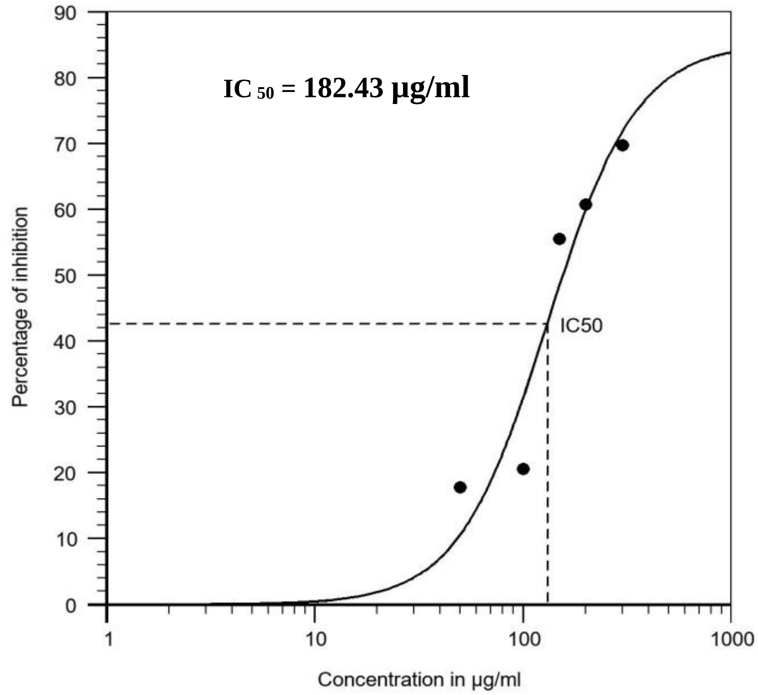


Fig.5. α - Glucosidase inhibitiory activity of rhizome of *Costus speciosus* at varying concentrations

IC 50 = 279.32 µg/ml

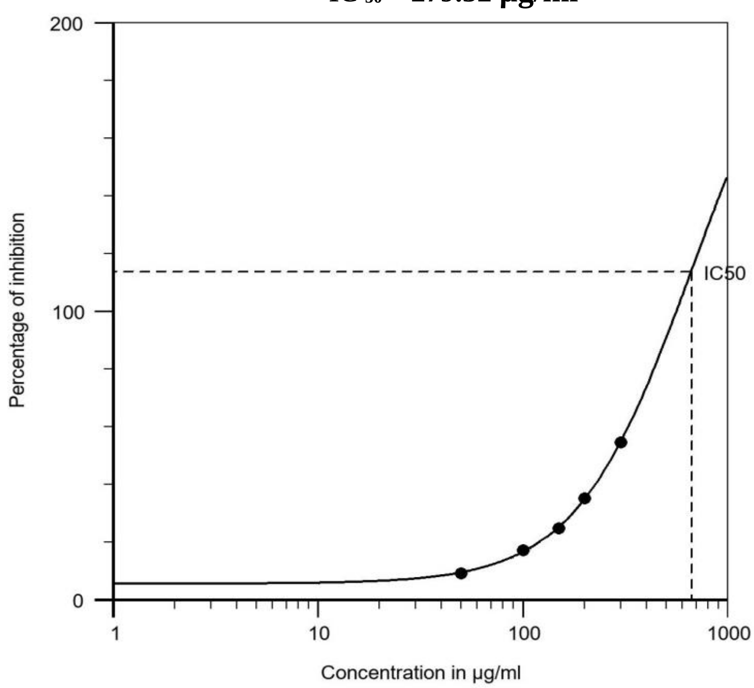


Fig.6. α - Glucosidase inhibitiory activity of Acarbose at varying concentrations

Table.4. Antidiabetic activity of *Costus pictus* and *Costus speciosus* using α - Amylase inhibitory assay

Concentration of plant extracts	*Costus pictus*		*Costus speciosus*		OD Values of Acarbose
	OD Values of leaf extract	OD Values of rhizome extract	OD Values of leaf extract	OD Values of rhizome extract	
100 µg/ml	0.109±0.001	0.066±0.007	0.141±0.012	0.267±0.006	0.312±0.012
200 µg/ml	0.149±0.007	0.083±0.011	0.199±0.002	0.437±0.010	0.285±0.010
300 µg/ml	0.224±0.010	0.1±0.009	0.284±0.001	0.587±0.04	0.259±0.009
200 µg/ml	0.275±0.09	0.108±0.010	0.327±0.006	0.776±0.008	0.223±0.008
300 µg/ml	0.315±0.006	0.131±0.008	0.391±0.009	0.979±0.003	0.156±0.011

Table. 5. Percentage of α - Amylase inhibitory activity

Concentration µg/ml	Percentage of Inhibition (%)				
	Costus pictus		*Costus speciosus*		Acarbose
	Leaf extract	Rhizome extract	Leaf extract	Rhizome extract	
100 µg	9.74	62.64	0	0	14.52
200 µg	21.20	69.05	6.30	0.51	29.71
300 µg	35.82	71.35	18.62	24.74	55.44
400 µg	57.31	76.22	42.98	43.97	64.02
500 µg	68.77	81.09	59.60	65.77	82.51

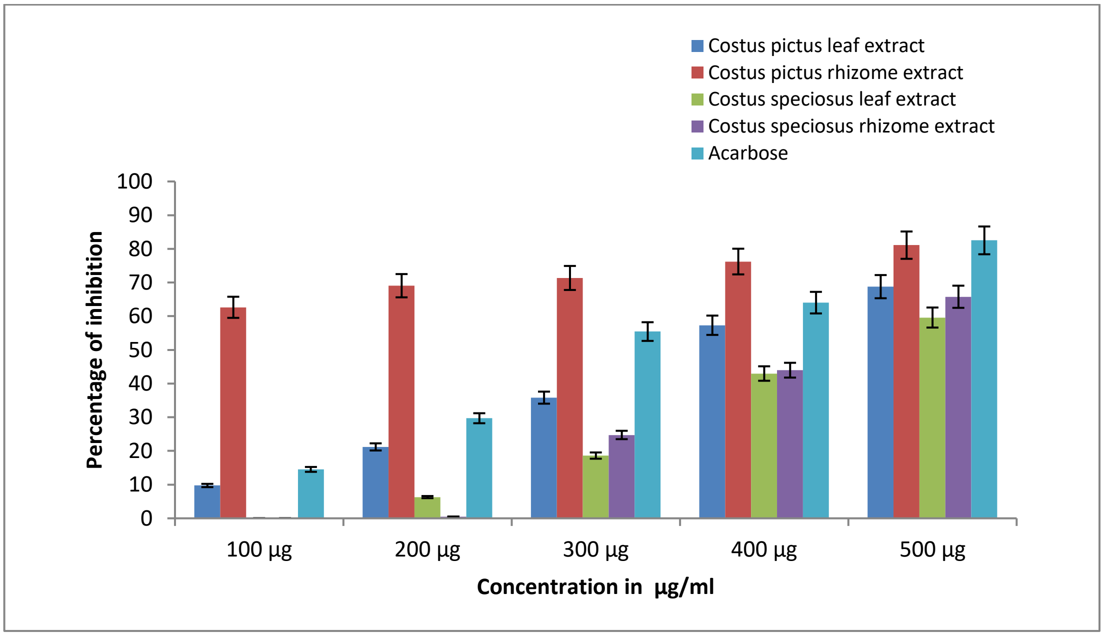


Fig.7: -α - Amylase inhibitiory activity of *Costus pictus* and *Costus speciosus*

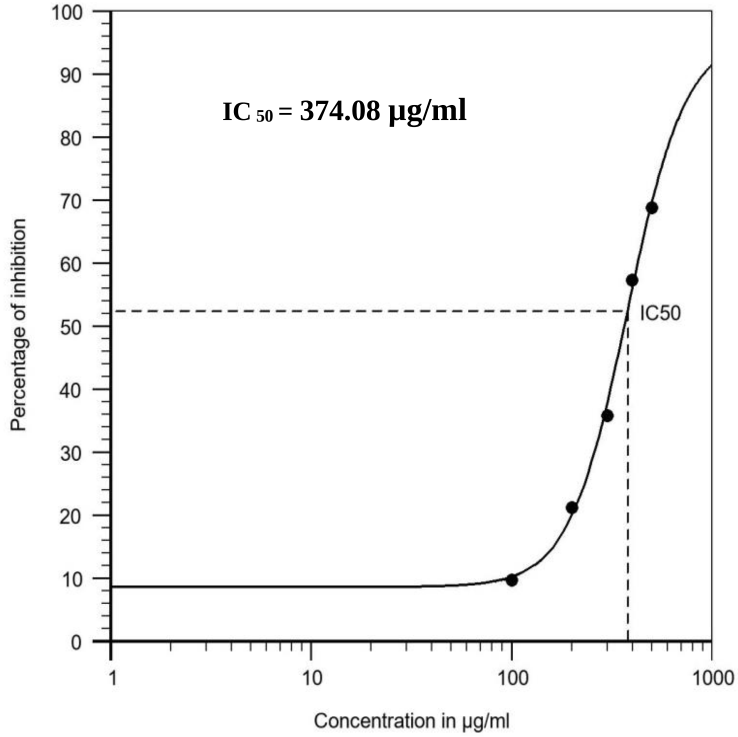


Fig.8. α - Amylase inhibitory activity of leaf extract of *Costus pictus* at varying concentrations

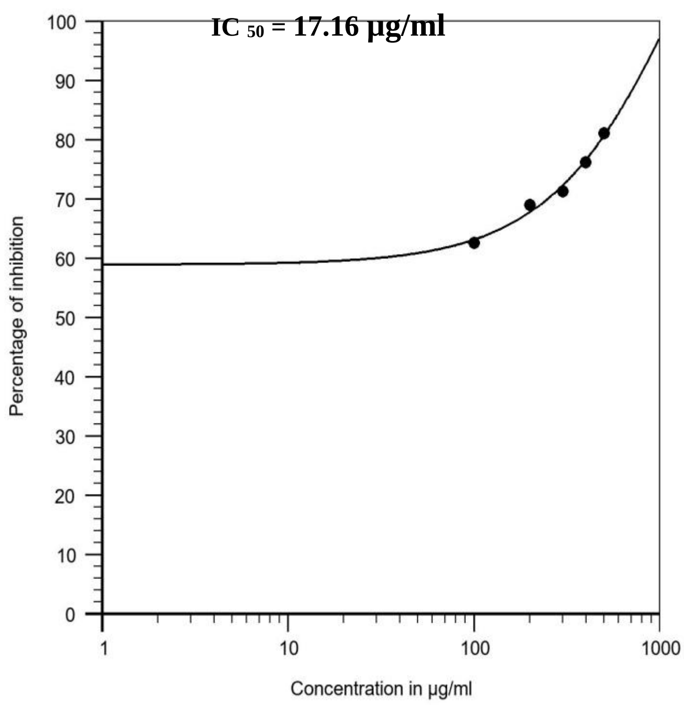


Fig.9. α - Amylase inhibitory activity of rhizome extract of *Costus pictus* at varying concentrations

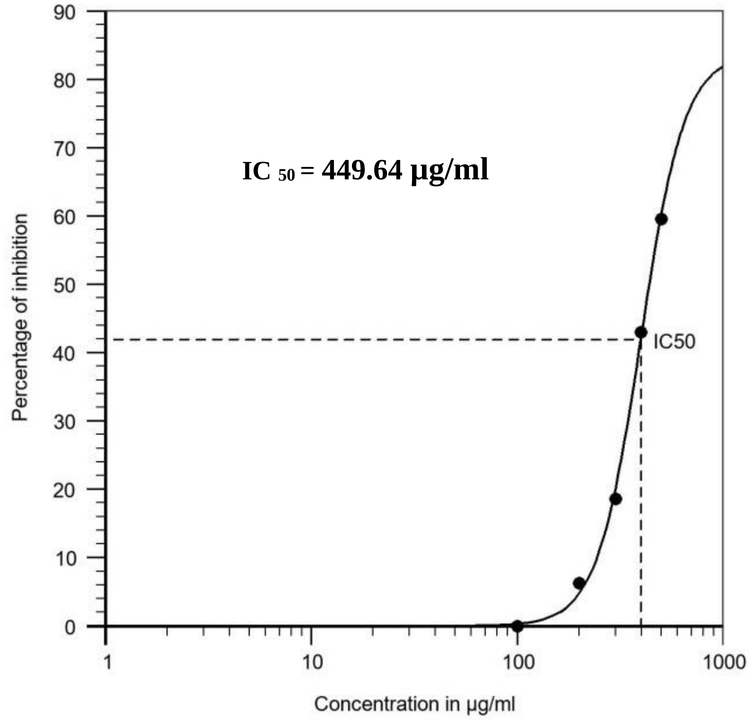


Fig.10. α - Amylase inhibitory activity of leaf extract of *Costus speciosus* at varying concentrations

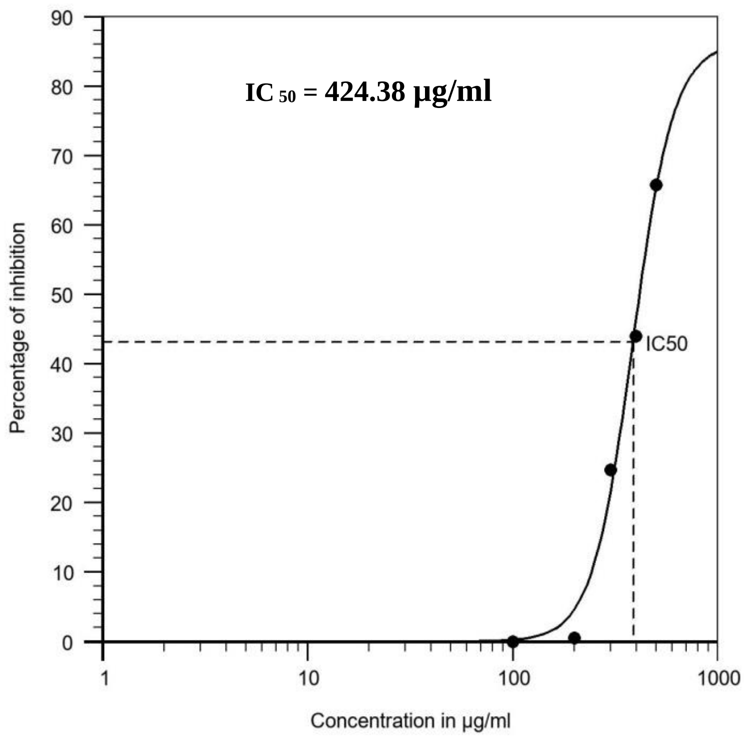
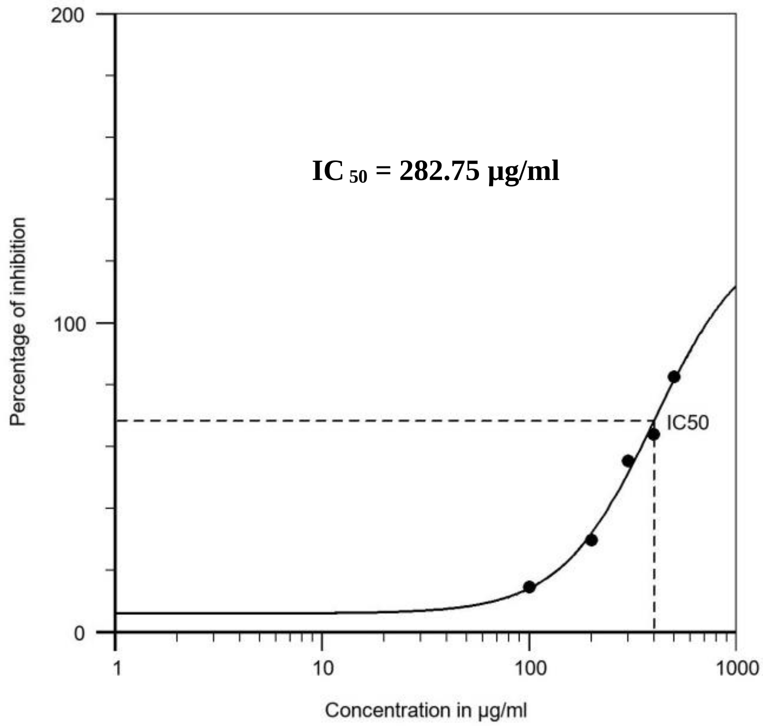


Fig.11. α - Amylase inhibitory activity of rhizome extract of *Costus speciosus* at varying concentrations



The data is indicated as the mean ± SEM; (n = 5). (P < 0.001)

Fig.12. α - Amylase inhibitory activity of acarbose at varying concentrations

4. DISCUSSION

Diabetes mellitus is one of the most common disorders that prevalent almost 6% of the world population and International Diabetes Federation's (IDF) estimated that 80% could be occurred in low- and middle-income countries in 2030. It is one of the six major causes of death and treated by hormone therapy (insulin) or by administering glucose-lowering agents such as alpha-glucosidase inhibitors, sulfonylureas, biguanides, and thiazolidinediones. Polyhedral formulation is well practiced against diabetes that has better and extended therapeutic potential allopathic medicines. Hence, the present study was planned to evaluate and compare four poly herbal formulations using well known *invitro* methods.

5. CONCLUSION

In this present study the antidiabetic activity was evaluated by using two important enzymes, alpha-amylase and alpha-glucosidase by *in vitro* methods. The higher inhibitory activity was observed in rhizome extract of *Costus pictus* and rhizome extract of *Costus speciosus*. Further detail investigation will be helpful for developing a potential antidiabetic drug from this herbal formulation.

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