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AMYLASE INHIBITORY ACTIVITY OF *COSTUS IGNEUS* LEAF EXTRACT

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ABSTRACT
Diabetis mlletus is one of the autoimmune diseases where the human population is at the receiving end. The disease affects large human population in the Indian subcontinent. The present study was aimed at inhibiting the enzyme amylase by the methanol leaf extract of *Costusigneus*. The inhibition assay was performed by Chromogenic DNSA method. The leaf methanol extract has displayed inhibition of 65.48, 35.72 and 3.57% at 500, 250 and 100 µg concentrations. Whereas, the standard Acarbose inhibited the enzyme at all concentrations screened. From the results it indicates that the α-amylase inhibition by the methanol extract of *C. igneus* is dose dependent.

KEY WORDS: *Diabetes milletus*, Phytochemical screening, Amylase activity, *Costusigneus*, DNSA method.

INTRODUCTION

Diabetes mellitus is a multifactorial disorder characterized by high glucose from increased hepatic glucose production, diminished in insulin production and impaired insulin action. It is disease of worldwide significance and increases their prevalence without any plateau. There are many drugs available to control and treat patient's diabetics, but total recovery is yet not possible. Conventional drugs treat diabetes by improving insulin sensitivity by increasing insulin production and decreasing the amount of glucose in blood. In addition to these adverse effects of drug treatments are not always satisfactory in maintaining normal level of blood glucose [1]. Hence, there is an urgent need of new products for this ailment. The Western Ghats is one of the eighteen biodiversity hotspots in the world and plants available in this part are very unique to this area. The area provides a potential source of the anti-diabetic plants used for the treatment of *Diabetes mellitus*. From the literature survey we came to know that low glucose effects of pharmacologically active components of plants in diabetes patients is assessing by the lowering affects on α-amylase (both salivary and pancreatic) plant components and various direct and indirect effects of different parameters responsible for the development of diabetes [2]. Amylases are one of the most important and oldest industrial enzymes. These comprise hydrolases,which hydrolyse starch molecules to fine diverse products as dextrins and progressively smaller polymers composed of glucose units. Large arrays of amylases are involved in the complete breakdown of starch. However, α-amylases which are the most in demand hydrolyse α-1, 4 Glycosidic bond in the interior of the molecule [3]. Recently, interest in substance of plant origin which promotes normalization of lipid metabolism under conditions of lipid pathology has increased [4]. The protective effects of plant products are due to the presence of several components, which have distinct mechanism of action, some of them are enzymes, proteins and other lower molecular weight compounds such as vitamins, carotenoids, Flavonoids.[4] Anthocynins and other phenolic compounds [5]. In view of the above facts *Costusigneus* Family: Costaceae, common name Fiery Costus or Spiral Flag is a species of herbaceous plant [6]. Has been considered for our investigation due to it's prolong use by the diabetic patients in this particular part of India. This has been eaten raw for the normalization of blood glucose level. The plant has large fleshy leaves. The undersides of these large, smooth, dark green leaves have light purple shade. The leaves are spirally arranged around the stem, forming attractive, arching clumps arising from underground rootstocks. The maximum height of these plants is about two to three feets.

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The flowers are orange in color and are beautiful, 1.5-inch diameter. Flowering occurs during the warm months and they appear to be cone-like heads at the tips of branches. The leaves of the plant to consume by drying and grinding powder of the leaves [7].

The methanolic and aqueous extracts of *C. igneus* at a dose of 200 mg/kg body weight reversed the diabetes-induced hyper lipidemia [8]. Alcoholic extract of *C. igneus* at the dose of 400 mg/kg (p. o.) had significantly decreased the levels of serum cholesterol, triglycerides, LDL in Triton-induced hyper lipidemic rats [9]. Oral administration of ethanolic extract of *C. igneus* rhizome at 200 mg/kg body weight to diabetic rats for 30 days induced a significant antioxidant effect. The bioactive compound quercetin and diosgenin present in the plant exhibited antioxidant activity, which was sufficient to reverse oxidative stress in liver, pancreas, and kidney of diabetic rats as well as to stimulate glycolytic enzymes and control gluconeogenesis in diabetic animals [10]. The methanolic extract of *C. igneus* showed maximum anti-bacterial activity against *gram-positive* and negative strains [11]. The isolated compound from the ethanolic extract of *C. igneus* showed moderate anti-bacterial and anti-fungal activity against *Staphylococcus aureus*, *Eschericia coli* and *Candida albican* [12].The aqueous extract of *Costus* stem and isolated compounds lupeol, and stigmasterol had an inhibitory effect on calcium oxalate urolithiasis, and its putative activity was confirmed by the promotion of formation of calcium oxalate dehydrate (COD) crystals and may possibly treat urinary stones by inhibiting the formation of calcium oxalate monohydrate (COM) crystals [13]. In the present study methanol leaf extract of *C. igneus* has displayed significant inhibition of enzyme at 500µg concentration and methanol could not inhibit any activity, significantly.

MATERIALS AND METHODS

Chemicals

The chemicals and reagents used were of analytical grade. Diastase (HiMedia), Mumbai, India.Methanol (HiMedia). Other chemicals such as potassium sodium tartarate, dinitrosalicylic acid and sodium hydroxide used for the study were of analytical grade.

Plant Material

The plant was collected in the month of February-March-2014 from Shakarayapattan town of Chikmagalur District, Karnataka state, India and was authenticated at the Herbarium Department of Botany, Kuvempu University, Shankaraghatta, Shimoga Dist. Karnataka State. India.

Preparation of Extract

Cold extraction method was employed to extract the plant material. Exactly weighed 650 gm of fresh leaves of the plant material was subjected for crushing with the help of a blender. The paste obtained was filtered with a muslin cloth. The filterate is evaporated and the residue was introduced into a flat bottom flask of one liter capacity and to it one liter of methanol was added and kept at room temperature with occasional swirriling with a time gap of about 15 min for about 48 hrs. After complete extraction, the solvent was removed by using a rotaevaporater under reduced pressure and controlled temperature of 40^{o}C. The extract thus obtained (3.846gm) was stored in refrigerator at -10^{o}C until further investigation.

Experimental Design

Method: The inhibition assay was performed using the chromogenic DNSA method [Miller, 1959]. The total assay mixture composed of 1400 µl of 0.05 M sodium phosphate buffer (pH 6.9), 50 µl of amylase (Diastase procured from HiMedia, Mumbai, Cat No. RM 638) and samples (Methanol extract) at concentration 100, 250 and 500 µg were incubated at 37°C for 10 min. After pre-incubation, 500 µl of 1% (w/v) starch solution in the above buffer was added to each tube and incubated at 37°C for 15 min. The reaction was terminated with 1.0 ml DNSA reagent, placed in boiling water bath for 5 min, cooled to room temperature and the absorbance measured at 540 nm. The control amylase represented 100% enzyme activity and did not contain any sample of analysis. To eliminate the absorbance produced by sample, appropriate extract controls with the extract in the reaction mixture in which the enzyme was added after adding DNS. The maltose liberated was determined by the help of standard maltose curve and activities were calculated according to the following formula.

$$\text{Activity} = \frac{\text{Conc. of maltose liberated X ml of enzyme used}}{\text{Mol. Wt. of maltose X incubation time (min)}}$$

One unit of enzyme activity is defined as the amount of enzyme required to release one micromole of maltose from starch per min under the assay conditions.

The inhibitory/induction property shown by the extract was compared with that of control and expressed as percent induction/inhibition.This was calculated according to the following formula

$$\%\ \text{inhibition/induction} = \frac{\text{Activity in the presence of compound}}{\text{Control activity}} \times 100$$

RESULTS

The assay performed by the DNSA method to inhibit the enzyme α-amylase by the methanol leaf extract indicated that the inhibition was indeed dose dependent. The result is represented in table-1 shows that the methanol extract at 500µg inhibit 65.84% where as the standard acarbose inhibited 100% at 100µg (minimum concentration). The methanol extract inhibited around 35.72% at 250µg and showed almost nil inhibition at 100µg concentration. The control amylase showed no inhibition. Similarly methanol used as solvent did not show α-amylase inhibition. This clearly indicates that inhibition activity is indeed due to the crude methanol extract which is dose dependent.

Table-1 Showing the Assay Data

Sample	OD at 540 nm	Concentration of Maltose liberated (µg)	Activity (µmoles/ml/min)	% of Activity	% of Inhibition
Control	1.15	70	0.0194	100.00	0.00
MI (100 µg)	1.12	67.5	0.0187	96.43	3.57
MI (250 µg)	0.85	45	0.0125	64.28	35.72
MI (500 µg)	0.6	24.17	0.0067	34.52	65.48
Acarbose (100 µg)	0	0	0.0000	0.00	100.00
Acarbose (250µg)	0	0	0.0000	0.00	100.00
Acarbose (500 µg)	0	0	0.0000	0.00	100.00
Methanol (10 µl)	1.14	69.17	0.0192	98.81	1.19
Methanol (25 µl)	1.12	69.17	0.0192	98.81	1.19
Methanol (50 µl)	1.1	68.33	0.0190	97.62	2.38

*OD= optical density

Figure 1. Graphical representation of optical density Vs concentration of maltose

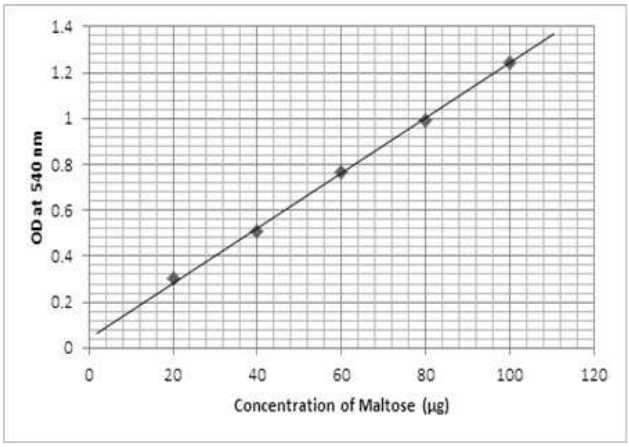
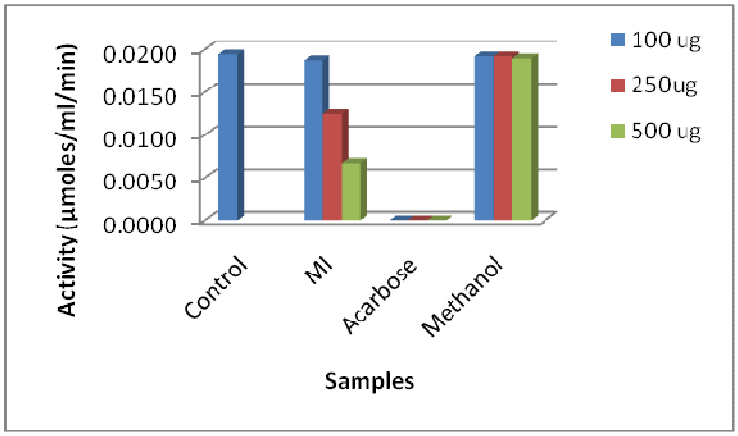


Figure 2. Graph showing the comparative amylase inhibition analysis of *Costusigneus* extract, methanol and Acarbose



DISCUSSION

The α-amylases are calciummetalloenzymes, completely unable to function in the absence of calcium. By acting at random locations along the starch chain, α-amylase breaks down long-chain carbohydrates, ultimately yielding maltotriose and maltose from amylose or maltose, glucose and "limit dextrin" from amylopectin. Because it can act anywhere on the substrate, α-amylase tends to be faster-acting than β-amylase. In animals, it is a major digestive enzyme, and its optimum pH is 6.7–7.0 N [14].

The role of α-amylase is to cleave the large starch molecules into smaller fragments of sugars which in terms utilized by the brain as its energy source, because the large molecules like starch cannot pass through blood brain barrier to overcome from this the problem larger starch molecules converted into smaller fragments in order to pass through the blood brain barrier. The excess conversion of starch to sugars (remaining sugar moieties which left by brain) will increase the sugar level in blood, the role of insulin come in action here it will order cells to metabolize the excess sugar moieties and store as energy sources i. e. glycogen. This cycle will continuously going on in health person. In some cases the excess activity of amylase enzyme will leads to hyperglycemia (high sugar level in blood). Due to the deficiency of insulin or resistance to insulin hyperglycemia arises.[14]To overcome this problem to control sugar level in blood we can inhibit the activity of amylase enzyme with the help of our extract at higher concentration.The inhibition assay was carried out by using the chromogenic DNSA method [15].In this methodpreparing the DNSA reagent. First step of experiment we have taken 50µl of α-amylase, 1400 µl of sodium phosphate buffer and different concentration of samples (methanol extract) such as 100 µg, 250 µg and 500 µg were incubated at 37^{o}C for about 10min. while incubation period the methanol extract will bind the α-amylase, it converts into inactive α-amylase. In second step 500µl of 1% (w/v) starch solution was added to each tube containing enzyme and further incubated at 37^{o}C for 15min. If the methanol extract having the potency to inactivate the α-amylase enzyme then the starch molecule cant undergoes cleavage at 1, 4 glycosidic linkage or otherwise it will break the 1, 4 glycosidic linkage.

Once it will cleave the 1, 4 glycosidic linkages it forms the corresponding glucose moieties in the medium. In termination step 1ml of DNSA reagent was added and placed in a boiling water bath for 5min, cooled to room temperature and the absorption was measured at 540nm. If the α-amylase is in active condition starch molecule break down into glucose.

In this step DNSA acts as an oxidizing agent it oxidizes the moiety and itself undergo reduction. The oxidized glucose molecules develop the wine red color, and the concentration can be estimated by spectrophotometry. Based upon the concentration of the color we can analyze the percentage of activity and percentage inhibition of α-amylase with methanol extract.

The present investigation shows that the methanol extract at 500µg inhibit 65.84% where as the standard acarbose inhibited 100% at 100µg (minimum concentration). The methanol extract inhibited around 35.72% at 250µg and showed almost nil inhibition at 100µg concentration. This clearly indicates that inhibition activity is indeed due to the crude methanol extract which is dose dependent.

CONCLUSION

The regular consumption of insulin plant leaves in conjunction with other modalities of treatment has effectively provided glycaemic control in diabetes, the risk of diabetic complications was avoided and no adverse effects due to the consumption of insulin plant leaves. The present investigationwe conclude that the methanolic crude extract of *Costusigneus* or insulin plant have shown significant amount of inhibition of amylase enzyme, which is may be due to presence of phytochemicals (Alkaloids, Flavonoids, Tannins, and Steroids) present in a methanolic extract of *Costusigneus*. Hence, the inhibition is due to the presence of any one kind of phytochemicals or combination of phytochemicals. The greater inhibition shown at higher concentration (500 µg compare to 250 and 100 µg) it helps to prevent the hyperglycemia problem.

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REFERENCE

1. Prabakar P. K. Doble M. Mechanism of Action of Natural Products Used In the Treatment of Diabetes Mellitus, *Chinese Journal of Integrative Medicine,* 17(8), 2011, 563-574.
2. Raman B. V. Krishna A. N. V. NarashimRao B. PardhaSaradhi M. BasaveswaraRao M. V. Plants with Antidiabetic Activities and Their Medicinal Values, *International Research Journalof Pharmacy,* 3(3), 2012, 11-15.
3. Gupta R. Gigras P. Mohapatra H. Goswami V. Chauhan B. Cloning and overexpression of raw starch digesting α-amylase gene from Bacillus subtilis strain AS01a in Escherichia coli and applicationof the purified recombinant -amylase (AmyBS-I) in raw starch digestion and baking industry, *Journal of Molecular Catalysis B, Enzymatic*, 00, 2003, 1-18.
4. Zhang H. Y. Wang L. F. Theoretical elucidation on structure-antioxidant activity relationships for indolinonichydroxylamines, *Bioorganic Medicinal Chemistry letter*, 12, 2002, 225-227.
5. Sanchez-Moreno C. Larrauri J. A. Saura-Calixto F. A procedure to measure the antiradical efficiency of Polyphenols, *Journal of Science of Food* and *Agriculture*, 76(2), 1998, 270-276.
6. Krishnan K. Vijayalaxmi N. R. Helen A. Beneficial effects of Costusigneus and dose-response studies in streptozotocin induced diabetic rats, *Asian Journal of Pharmaceutical and Clinical Research,* 2, 2011, 42-46.
7. Devi V. D. Urooj A. Isolation, Characterization and Quantification of Diosgenin from Costusigneus, *Indian Journal* of *Experimental Biology*, 46(8), 2008, 614-616.
8. Mani P. Kumar A. R. Bastin T. M. Jenifer S. Arumugam M. Arumugam M. Comparative evaluation of extracts of C. igneus (or C. pictus) for hypoglycemic and hypolipidemic activity in alloxan diabetic rats, *International Journal of Pharmaceutical Technology*, 2, 2010, 183-195.
9. Chacko N. Shastry C. S. Shetty P. Shyamma P. souza U. D. Maulika P. Anti hyperlipidemic activity of Costusigneus in Triton X-100 induced hyperlipidemic rats, *International Journal of Pharmaceutical and Chemical Sciences,* 1, 2012, 813-818.
10. Kalailingam P. Sekar A. D. Samuel J. S. Gandhirajan P. Govindaraju Y. Kesavan M. The efficacy of Costusigneus rhizome on carbohydrate metabolic, hepatoproductive and antioxidative enzymes in steptozotocin-induced diabetic rats, *Journal of Health Science*, 57(1), 2011, 37-46.
11. Gothandam K. M. Aishwarya R. Karthikeyan S. Preliminary screening of antimicrobial properties of few medicinal plants, *Journal of Phytology*, 2, 2010, 1-6.
12. Saraswathi R. Upadhyay L. Venkatakrishnan R. Meera R. Devi P. Isolation and biological evaluation of steroid from stem of Costusigneus, *Journal of Chemical and Pharmaceutical Research,* 2(5), 2010, 444-448.
13. Manjula K. Pazhanichamy K. Kumaran S. Eevera T. Keefe C. D. Rajendran K. Growth characterization of calcium oxalate monohydrate crystals influenced by Costusigneusaqueous stem extract, *International Journal of Pharmacy* and *Pharmaceutical Sciences*,4(1), 2012, 261-270.
14. Das S. Singh S. Sharma V. Soni M. L. Isolation and medium optimization for amylase producing bacterial strain isolated from potato field of Bhatinda, Punjab, India, *International Journal of Pharma, Bio, and Sciences*,2(1), 2011, 486-496.
15. Miller G. L. Use of dinitrosaiicyiic acid reagent for determination of reducing sugar,*JournalofAnalytical Chemistry*, 1959, 31(3), 426-428.