

In Vitro Evaluation of Anti-Diabetic Activity of Leaf and Callus Extracts of *Costus pictus*

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Abstract: Herbal medicines are promising choice over synthetic drugs. *Costus pictus* is commonly known as 'Spiral ginger' or 'Step ladder' or 'Insulin plant' as its leaves are proved to produce antidiabetic effects. In present study callus culture of *Costus pictus* was initiated from the leaf explants. The leaf and callus extract of *Costus pictus* in four different solvents viz ethanol, acetone, aqueous and ethyl acetate were assessed for their possible effect on glucose diffusion across the dialysis membrane, a model system for glucose diffusion across the gastrointestinal tract. The ethyl acetate leaf and callus extract significantly decreased glucose movement as compared to other extract. Another approach to prevent diabetes is to control the abnormal postprandial increase of blood glucose level. This can be achieved by the inhibition of carbohydrate hydrolyzing enzymes like α -amylase and α -glucosidase. In present study the satisfactory inhibitory effect on α -amylase extracts from fresh *Solanum tuberosum* and α -glucosidase extracted from *Sorghum vulgare* seeds was investigated. The leaf and callus extract of *Costus pictus* in four different solvents viz ethanol, acetone, aqueous and ethyl acetate studied in vitro shows good inhibitory activity on these enzymes. Higher α -amylase inhibitory effect of about 77.53% was shown by callus extract in ethyl acetate and leaf extract in ethanol. In case of α -glucosidase ethanol callus extract showed highest inhibitory effect of about 86.94%. The results of this study may be useful to find out the mode of action of this leaf and callus extracts of *Costus pictus* as α -amylase and α -glucosidase enzyme inhibitors and to know the possible mechanism for antidiabetic activity.

Keywords: α -amylase, α -glucosidase, antidiabetic, Callus, *Costus pictus*.

1. Introduction

Diabetes mellitus is a clinical syndrome characterized by inappropriate hyperglycemia caused by deficiency of insulin at the cellular level [1]. It is associated with disturbances of carbohydrate, fat and protein metabolism. Since oral hypoglycemic agents cause side effects, there is a growing interest in herbal remedies for the treatment of diabetes mellitus [2]. Control of plasma glucose concentrations is vital to decrease the incidence and severity of long-term diabetic complications [3]. Antihyperglycemic activities can be explained by the ability of water soluble plant components to increase glucose transport and metabolism in muscle and/or to stimulate insulin secretion [4-10]. One therapeutic approach for treating diabetes is to decrease postprandial hyperglycemia. This can be achieved by delaying the absorption of glucose through the inhibition of carbohydrate hydrolyzing enzymes α -amylase and α -glucosidase in the digestive tract. α -glucosidase inhibitors can retard the liberation of glucose from dietary complex carbohydrates and delay glucose absorption, resulting in reduced postprandial plasma glucose levels and suppress postprandial hyperglycemia [11]. α -amylase inhibitors inhibit the action of α -amylase enzyme leading to a reduction in starch hydrolysis which shows beneficial effects on glycemic index. α -amylase and α -glucosidase inhibitors are drug design targets in the development of compounds for the treatment of diabetes [12] [13]. *Costus pictus* is one of the medicinally important plants belonging to the *Zingiberaceae* family [14]. It is commonly known as 'Spiral ginger' or 'Step ladder' or 'Insulin plant' as its leaves are proved to produce antidiabetic effects [15]. In India, it is grown in gardens as an ornamental plant in Kerala, where fresh raw leaves are eaten by diabetic people [16] [17].

The present study was undertaken to investigate the effects of antidiabetic plant on glucose movement across dialysis

membrane into external solution, which is a convenient model for assessing factors affecting glucose absorption in vitro. Further study was conducted to find out the α -amylase and α -glucosidase inhibitory activity of this plant to know the possible mechanism of antidiabetic activity of *Costus pictus*.

2. Material and Methods

2.1 Plant material

Healthy and young plants of *Costus pictus* (3- 6 months old) were purchased from Kerala and maintained in Paradise nursery, Nashik.

2.2 Callus culture of *Costus pictus*

The leaf explants were washed thoroughly under running tap water for 2 h. The explants were then sterilized with 0.1% HgCl₂ for 10 min. It was then given treatment with 70% ethanol for 2 s. The explants were rinsed with sterile distilled water for 5-6 times and were used for inoculation. Leaf (10-12mm²) was cut with the help of sterile forceps and scalpel. The explants were aseptically transferred into the 1/2 strength MS medium containing IAA + BAP (1.5mg/L). The explants were incubated under 16 h photoperiod of 2000-3000 lux at 25±2°C, until callus was induced [18].

2.3 Plant material and extract preparation

Leaves of *Costus pictus* plant and leaf callus were oven dried at 100°C for 3-4 days. Powdered leaves (0.5gm) and leaf callus (0.5gm) was mixed with four different solvents like Ethanol, acetone, aqueous, ethyl acetate (20ml) and refluxed for 2 h and left overnight. The mixture was then filtered and the solvent was evaporated. The dried powder was dissolved in distilled water and stored at 40°C.

2.4 In vitro antidiabetic activity (By glucose diffusion inhibitory study)

This is the in vitro technique used to evaluate the effects of plant and callus extract on glucose movement. In control experiment, 1ml of 0.15M sodium chloride containing 0.22M D-glucose was introduced in dialysis bag and sealed at each end. Test experiment consisted of dialysis bag into which 1ml of 2.5g/100ml of extract and 1ml of 0.15M sodium chloride containing 0.22M D-glucose was added [19] [20]. The dialysis bag was placed on magnetic stirrer and kept at room temperature. The appearance of glucose in external sodium chloride (0.15M) solution was measured by dinitrosalicylic acid method over the incubation period of 1h, 3h, 5h, 24h and 27h. 1ml of sample was taken to which 1ml of dinitrosalicylic acid was added, boiled for 15 minutes and absorbance was recorded at 540 nm [21].

2.5 Inhibition assay for α -amylase

Alpha amylase was extracted from fresh potato tuber (*Solanum tuberosum*), method describe by May lay fan 1975 [22]. A reaction mixture containing 500 μ l of plant and callus extract, 500 μ l 0.02M sodium phosphate buffer (pH 6.9 with 0.006M sodium chloride) and 500 μ l α -amylase enzyme solution was incubated at 25°C for 10min. After pre-incubation, 500 μ l of 1% starch solution 0.02M sodium phosphate buffer (pH 6.9 with 0.006M sodium chloride) was added to each tube at 5s intervals. The reaction mixture was then incubated at 25°C for 10min. The reaction was stopped with 1.0 ml dinitrosalicylic acid colour reagent. The test tubes were then incubated in boiling water bath for 15min and cooled to room temperature and absorbance was measured at 540nm. Each experiment was performed in duplicates [23].

$$\% \text{ Inhibition} = \frac{[(A_{540} \text{ Control} - A_{540} \text{ Extract})] \times 100}{A_{540} \text{ Control}}$$

2.6 Inhibition assay for α -glucosidase

Alpha glucosidase was extracted from *Sorghum vulgare* seeds, method describe by Chikezie I. Ovvuama 1999 [24]. A reaction mixture containing 500 μ l of plant extract, 500 μ l 0.02M sodium phosphate buffer (pH 6.9 with 0.006M sodium chloride) and 500 μ l α -glucosidase enzyme solution was incubated at 25°C for 10min. After pre-incubation, 500 μ l of 1% starch solution 0.02M sodium phosphate buffer (pH 6.9 with 0.006M sodium chloride) was added to each tube at 5s intervals. The reaction mixture was then incubated at 25°C for 10min. The reaction was stopped with 1.0ml dinitrosalicylic acid colour reagent. The test tubes were then incubated in boiling water bath for 15 min and cooled to room temperature and absorbance was measured at 540 nm. Each experiment was performed in duplicates [23] [25].

$$\% \text{ Inhibition} = \frac{[(A_{405} \text{ Control} - A_{405} \text{ Extract})] \times 100}{A_{405} \text{ Control}}$$

3. Results

3.1 In vitro antidiabetic activity by glucose diffusion inhibitory study

The ethyl acetate leaf and callus extract was found to be more potent inhibitor of glucose movement than other extracts showing lowest mean glucose concentration in external solution of $275 \pm 7.071 \mu\text{g/ml}$ and $200 \pm 14.142 \mu\text{g/ml}$ respectively at the end of 27 h. The glucose conc. in external solution in leaf and callus extract in the decreasing order was ethyl acetate > ethanol > aqueous > acetone. After 27 h without leaf and callus extract (control), glucose movement out of dialysis had reached a plateau with a mean glucose concentration in the external solution was $420 \pm 14.142 \mu\text{g/ml}$ as shown in Figure 1 and Figure 2.

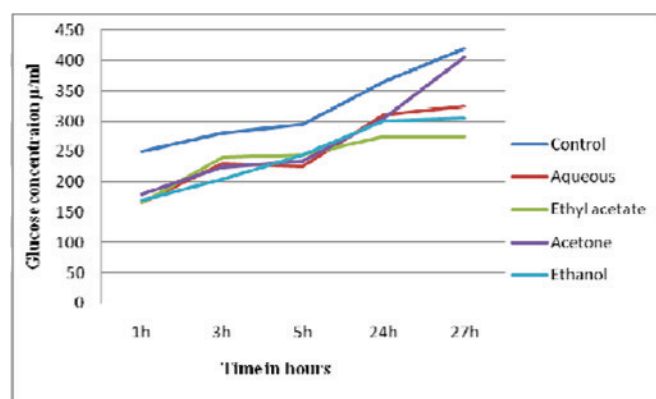


Figure 1: Effect of 2.5gm/100ml leaf extracts in different solvents of *Costus pictus*, on the movement of glucose diffusion out of dialysis tube

Values are mean of two observation; values are expressed as mean \pm Standard deviation.

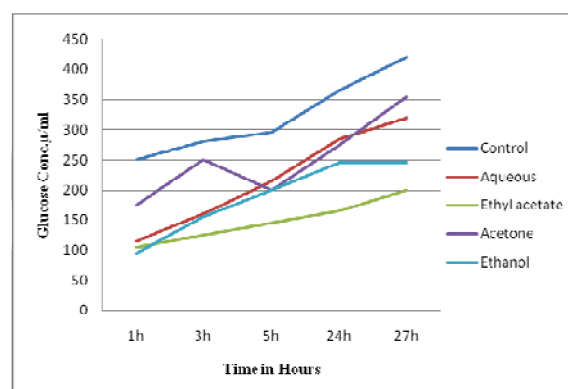


Figure 2: Effect of 2.5gm/100ml callus extracts in different solvents of *Costus pictus*, on the movement of glucose diffusion out of dialysis tube

Values are mean of two observation; values are expressed as mean \pm standard deviation.

3.2 Screening of leaf and callus extract on α -amylase inhibiting activity

The four extract (Ethanol, acetone, aqueous, ethyl acetate) of *Costus pictus* leaves and callus showed good inhibitory activity for α -amylase. As shown in Figure 3, among the four extract tested callus in ethyl acetate and leaf in ethanol

extract showed higher inhibitory effect of about 77.53% when compared with other extract. The inhibitory effect of leaf and callus extracts decreased in the order of: Ethanol> acetone> aqueous> ethyl acetate and ethyl acetate> aqueous> acetone> ethanol respectively.

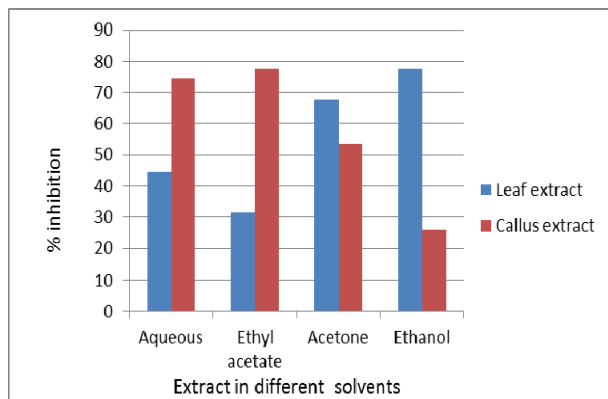


Figure 3: The enzyme inhibitory activity of different extracts of *Costus pictus* leaf and callus on α -amylase.

3.3 Screening of leaf and callus extract on α -glucosidase inhibiting activity

Among the four extract tested ethanol callus extract showed higher inhibitory effect of about 86.94 % when compared with other extract as shown in Figure 4. The inhibitory effect of leaf and callus extracts decreased in the order of: ethyl acetate>acetone>ethanol > aqueous and ethanol>aqueous> ethyl acetate> acetone respectively.

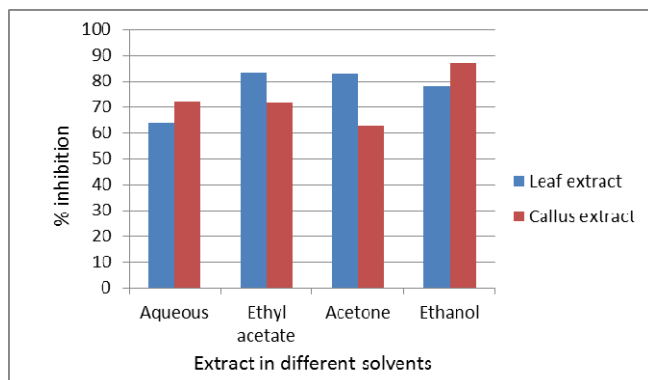


Figure 4: The enzyme inhibitory activity of different extracts of *Costus pictus* leaf and callus on α -glucosidase.

4. Discussions

4.1 In vitro antidiabetic activity

Diabetes mellitus is debilitating and life threatening disorder with increasing incidence throughout the world. Antihyperglycemic activities of most effective plants were in part explained by ability of phytoconstituents to increase glucose transport and metabolism in muscle and /or to stimulate insulin secretion. In vitro inhibitory activity can be related to in-vivo activity. The data obtained from in-vitro inhibitory activity can be used in preclinical animal studies. These studies are useful to isolate the phytoconstituents responsible for antidiabetic activity.

The effect of various extracts on glucose diffusion inhibition was tested. At the end of 27 h glucose movement of control

(without plant extract) in external solution has reached a plateau with mean glucose concentration above $420 \pm 14.142 \mu\text{g/ml}$. It was evident from graph that the ethyl acetate and ethanol leaf and callus extracts were found to be potent inhibitors of glucose diffusion. The ethyl acetate leaf and callus extract was found to be more potent than other extracts showing lowest mean glucose conc. of $275 \pm 7.071 \mu\text{g/ml}$ and $200 \pm 14.142 \mu\text{g/ml}$ respectively at the end of 27 h.

4.2 Inhibitory activity of α – amylase and α – glucosidase

In diabetes, hyperglycemia is condition characterized by abnormal postprandial increase of blood glucose level. In present study the satisfactory inhibitory effect on both enzymes support the use of this plant leaves as munching dietary supplement for treatment of diabetes. The results of these studies are useful to find out the mode of action of this leaf and callus extracts as α -amylase and α -glucosidase enzyme inhibitors and to know the possible mechanism of antidiabetic activity of *Costus pictus*.

4.2.1 Screening of leaf and callus extract on α -amylase inhibiting activity

The four extract-aqueous, ethyl acetate, acetone, alcohol of leaf and callus showed good inhibitory effects for α -amylase. Among the four extract tested ethyl acetate callus extract showed higher inhibitory effect of about 77.53% when compared with other extract. The inhibitory effect of leaf and callus extracts decreased in the order of: ethanol> acetone> aqueous> ethyl acetate and ethyl acetate> aqueous> acetone> ethanol respectively.

4.2.2 Screening of leaf and callus extract on α -glucosidase inhibiting activity

The four extract-aqueous, ethyl acetate, acetone, alcohol of leaf and callus showed good inhibitory effects for α -glucosidase. Among the four extract tested alcohol callus extract showed higher inhibitory effect of about 86.94% when compared with other extract. The inhibitory effect of leaf and callus extracts decreased in the order of: Ethyl acetate> acetone> ethanol> aqueous and ethanol> aqueous> ethyl acetate> acetone respectively. Nair et al [23] also describe the inhibitory activity of α – amylase and α – glucosidase in *Artocarpus heterophyllus*, *Artocarpus altilis*, *Piper betel* and *Cinnamomum zeylanicum* in methanolic extract.

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