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# PURIFICATION AND CHARACTERIZATION OF ANTI-HYPERGLYCEMIC BIOACTIVE MOLECULE FROM *COSTUS PICTUS* D. DON

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**Keywords:**

*Costus pictus*, Diabetes mellitus, β-Sitosterol-3-O-β-D-glucoside, Daucosterol, HPTLC

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**ABSTRACT:** *Costus pictus* D. Don., commonly known as the 'Insulin plant' is being used as a natural remedy to treat diabetes mellitus. The present study is focused to identify the chemical constituent responsible for the anti-diabetic activity of *Costus pictus*. The methanol extract was prepared from the fresh leaves of *Costus pictus* and washed with water to remove excess oxalic acid content present to get the non-toxic water-insoluble extract (CWI). The CWI was fractionated into low polar (CWI-LP), medium polar (CWI-MP) and high polar (CWI-HP) fractions by silica gel column chromatography. CWI and CWI-HP fraction showed prominent dose-dependent glucose tolerance effects in rats by GTT whereas the other fractions did not show any effect. Phytochemical analysis of CWI-HP resulted in isolation and characterization of the active compound present, namely β-Sitosterol-3-O-β-D-glucoside (daucosterol). The compound was purified by silica gel column chromatography and identified using various spectroscopic techniques, mainly through 1D and 2D NMR, HRMS. Since daucosterol is unstable in acidic pH, the enteric coating was performed on the molecule and CWI-HP fraction. Daucosterol and CWI-HP fraction were evaluated in rats after enteric coating at different doses and found to have significant hypoglycemic activity at a very low dose compared to uncoated compound and fraction. HPTLC estimation revealed that the CWI extract of *Costus pictus* is a rich source of daucosterol. This is the first effort to identify the active principle from *Costus pictus*.

**INTRODUCTION:** In this century, diabetes mellitus (DM) has been recognized as an issue of global public health and considered a "modern-day epidemic". It has become the most common metabolic disease which not only has a negative impression on the patient's quality of life but also places a huge financial burden on health care systems throughout the world [1].

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DM is a heterogeneous disorder characterized by hyperglycemia due to the relative deficiency of insulin or its resistance. This metabolic disorder results in abnormal carbohydrate, fat, and protein metabolism [2]. DM is managed by reducing oxidative stress, controlling and stabilizing blood glucose levels, and normalizing lipid metabolism disturbances. Untreated Type 2 DM greatly increases the risk of developing many complications like cardiovascular diseases, coronary artery disease, neuropathy, renal failure, retinopathy and blindness [3].

Polyphenolic compounds derived from plants possess a wide range of biological properties and are gaining significant interest in the elucidation of

the biological role in current years. A lot of plant-derived active principles containing numerous natural chemical compounds have demonstrated activity consistent with their possible use in the treatment and maintenance of DM [4]. *α*-amylase and *α*-glucosidase inhibitors are targets in the development of drugs for the treatment of diabetes, obesity, and hyperlipaemia [5]. *Costus pictus* D. Don (*Costaceae*), commonly known as 'insulin plant' is widely studied scientifically for hypoglycemic activity [6]. The leaves of *Costus pictus* were extensively used for the management of sugar level by the people in Kerala, India [7, 8]. Hypoglycemic effect of *Costus pictus* alcoholic extract and its fractions have been reported earlier in alloxan-induced diabetic rats [9, 10, 11]. Histopathological analysis revealed increase in pancreatic beta cells of the animals treated with *Costus pictus* extract [10]. Aqueous extract of aerial part of *Costus pictus* induces a natriuretic response similar to that of furosemide [12]. It has been reported that *Costus pictus* extract inhibited carbohydrate hydrolyzing enzyme-like α-amylase and α-glucosidase [13]. Gerrish *et al*., found that the glucose-lowering effect of *Costus pictus* was associated with the potentiation of insulin release from islets of the pancreas and peripheral utilization of glucose [14]. A few other studies also confirm these findings and revealed hypoglycemic and anti-diabetic potential of *Costus pictus* [15, 16, 17].

Though many studies have been conducted to explore the anti-diabetic effects of *Costus pictus*, studies to find out the active principles responsible for hypoglycemic effects are still lacking. The present study was undertaken with the objective of isolation and characterization of the anti-diabetic principle present in *Costus pictus* leaves. An attempt was made to explore the possibility of enteric coating on active fractions and purified molecules to protect it from stomach acidic pH and hence to improve biological benefits at low doses. HPTLC estimation of the active constituent present in the extract was also carried out. This is the first report on the identification of the secondary metabolite responsible for anti-diabetic activity present in *Costus pictus* leaves.

## MATERIALS AND METHODS:

**Plant Material:** Fresh plant material was collected from a cultivated farm at Angamaly, Kerala, India, and identified by comparison with the reference sample (CP-2001 and accession number MH: 173773, Madras Herbarium, Botanical Survey of India, Southern Regional Centre, Coimbatore, Government of India).

**Animals:** Adult male albino rats (Sprague-Dawley), each weighing 200-250 g were purchased from Small Animals Breeding Station, Mannuthy, Kerala and housed in the animal research facility at Arjuna Natural Private Ltd. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and approved by the Institutional Animals Ethics Committee (IAEC) of Arjuna Natural Private Ltd., Kochi, Kerala (1524/PO/RcBi/S/11/CPCSEA). The rats were maintained at 22-24°C, with 65% relative humidity with light and dark periods at 12-h light/12-h dark intervals. Animals had free access to food and water and were acclimatized for one week before the commencement of the experiment.

**Extraction:** Fresh leaves of *Costus pictus* (10 Kg) were crushed, filled in the Soxhlet extractor and extracted with methanol (30 L). The extraction was performed using an extractor with a reflux condenser. The bottom of the extractor was fitted with a polypropylene (100 microns) filter cloth. The mixture was refluxed for five hours at 80 °C and cooled. The residue and supernatants were separated through a polypropylene filter cloth using a centrifugal pump. The supernatants were pooled and concentrated in an Agitated thin film evaporator (ATFE) at a temperature of 70 °C to get the methanol extract. This was dried under vacuum to obtain the crude methanol extract (96 gm). The methanol extract was washed with water (1000 ml) at 50 °C and the water-soluble part was separated by centrifugation. The residue of centrifugation was collected and labeled as water-insoluble extract (CWI).

**Fractionation:** CWI was fractionated by using a flash chromatographic system, Biotage Isolera One. The silica gel particles of size 200-400 mesh were loaded into a KP-SIL100g SNAP cartridge and the column was primed (wet) with 20% ethyl acetate (EA) in hexane. Before loading to the column, CWI (10 gm) was impregnated with silica gel in 1:1

ratio. The impregnated fraction was transferred into the sample of the cartridge in a flash chromatographic system. After sample loading, column was initially eluted with 2 column volume (CV) of 20% EA in hexane followed by a gradient elution up to 100% EA followed by 10% methanol in EA [20% (2CV), 20-30% (1CV), 30% (1CV), 30-40% (1CV), 40% (1CV), 40-50% (1 CV), 50% (1CV) 50-75% (2 CV), 75% (2 CV), 75-100% (2 CV), 10 methanol in EA% (3 CV)]. Column eluted up to 50% EA in hexane was pooled as CWI-LP fraction, 50 to 100% EA was pooled as CWI-MP fraction and the fraction eluted at 10% methanol in EA was pooled as CWI-HP fraction.

**Glucose Tolerance Test (GTT) with CWI and Fractions:** After one week of acclimatization, 36 animals were randomly divided into 6 groups comprising of 6 animals in each group.

**Group I:** Vehicle
**Group II:** CWI (75 mg/kg)
**Group III:** CWI-LP (75 mg/kg)
**Group IV:** CWI-MP (75 mg/kg)
**Group V:** CWI-HP (75 mg/kg)
**Group VI:** Glibenclamide (1 mg/kg)

The animals were starved for 12 h and fasting blood sugar (FBS) was determined by collecting blood by pricking the tail vein and using a glucometer. Vehicle (1% polysorbate 80 in distilled water) was fed to the rats of group 1 at 10 ml/kg. Test samples/standard was administered orally to the respective groups of animals in the form of suspension made with the vehicle. A high dose of glucose (2.5 g/kg) was administered orally after 30 minutes of vehicle/extract/standard. Blood sugar was again measured with glucometer after 30 min, 1 h, 2 h and 3 h of glucose administration.

**Dosage study with CWI-HP:** Since CWI-HP was the active fraction of CWI, it was supposed to have glucose tolerance effects at lower doses as compared to CWI. The dosage study was conducted at 5, 10, 25, 50 and 75 mg/kg in rats by GTT. The procedure for GTT was the same as mentioned under 'GTT with CWI and fractions'.

**Isolation and Characterization of Chemical Constituents:** CWI-HP fraction was subjected to column chromatography over silica gel (60-120 mesh, 200 g) using a solvent system composed of ethyl acetate and methanol. The silica gel particles as a slurry in EA: Methanol 9.5:0.5 was loaded into a glass column (50 × 3 cm) and primed (wet) with the same solvent for a few minutes. Before loading to the column, CWI-HP fraction (2 gm) was impregnated with silica gel in a 1:1 ratio. The impregnated fraction was transferred into the column. After sample loading, the column was isocratically eluted with EA: Methanol 9.5:0.5. 10 ml fractions were collected in test tubes.

TLC was carried out for the fractions using the solvent system EA: Methanol; 9:1 and the fractions with identical profiles were pooled. Column fractions collected in tubes 28 to 34 contain a major compound in pure form (CWI-HP-P3) and the purity was identified by TLC (EA: Methanol 9:1, $R_f$ value 0.28). The purified compound was further subjected to various spectroscopic and spectrometric techniques especially 1D and 2D NMR analysis and HRMS analysis for its structure elucidation.

**HPTLC estimation of CWI-HP-P3:** Silica gel HPTLC plates (Kieselgel 60 F 254, 20 cm×20 cm, 0.2 mm thickness, Merck, Germany) were washed with methanol before use and kept at 60 °C for 30 min for the analysis. An authentic standard of CWI-HP-P3 from Sigma and CWI solutions were made in methanol. The samples at different concentrations were spotted by means of Camag Linomat V fitted with a Hamilton microlitre syringe. The plates were developed using ethyl acetate- methanol solvent system (90:10 v/v) in the CAMAG twin-trough glass chamber, previously saturated with the solvent for 30 min. The mobile phase compositions were chosen after testing different solvent systems of varying polarity.

After development, the plates were dried in an air oven at 50 °C and scanned using a TLC Scanner 3 (Camag) in absorbance-reflectance mode. The developed plate was also derivatized using Anisaldehyde- $H_2SO_4$ reagent and scanned using a TLC Scanner in white light. Various concentrations of isolated compounds and extracts were analyzed in triplicate. Quantity of CWI-HP-P3 was determined by means of calibration plots obtained through concentrations against peak area. Data processing was performed with WinCATS planar chromatography manager software (version 1.43).

**Enteric Coating of CWI-HP and CWI-HP-P3:** CWI-HP and CWI-HP-P3 were subjected to enteric coating to protect the active molecules from stomach acidic pH as per the published method with slight modification [18]. In brief, the CWI-HP and CWI-HP-P3 were granulated using 5% gum acacia and dried at 50 °C in a hot air oven. The granules were subsequently seal coated (5% weight gain) with HPMC (hydroxyl propyl methyl cellulose) based coating material and then enteric coating was performed (10% weight gain) with aqueous ethyl cellulose and sodium alginate-based dispersion in a PAM Glatt (GPCG 1.1) equipment.

**GTT with enteric-coated CWI-HP and CWI-HP-P3:** In order to study the glucose tolerance effect of the fractions and purified molecule, the animals were randomly divided into 10 groups comprising of 6 animals in each group.

**Group I:** Vehicle control
**Group II:** Enteric-coated CWI-HP (5 mg/kg)
**Group III:** Enteric-coated CWI-HP (10 mg/kg)
**Group IV:** Enteric-coated CWI-HP (20 mg/kg)
**Group V:** CWI-HP-P3 (5 mg/kg)
**Group VI:** CWI-HP-P3 (10 mg/kg)
**Group VII:** CWI-HP-P3 (20 mg/kg)
**Group VIII:** Enteric coated CWI-HP-P3 (5 mg/kg)
**Group IX:** Enteric-coated CWI-HP-P3 (10 mg/kg)
**Group X:** Enteric-coated CWI-HP-P3 (20 mg/kg)

The animals were starved for 12 h and fasting blood sugar (FBS) was determined by collecting blood by pricking the tail vein and using a glucometer. Vehicle (1% polysorbate 80 in distilled water) was fed to the rats of group 1 at 10 ml/kg. Uncoated test samples were administered orally to the respective groups of animals in the form of suspension made with the vehicle. Enteric-coated granules were fed directly using stainless steel cannula. Enteric-coated placebo granules were fed to the rats of the vehicle control group. A high dose of glucose (2.5 g/kg) was administered orally after 30 minutes of vehicle/uncoated extract. In the case of rats fed with enteric-coated samples, glucose (2.5 g/kg) was administered after 2 h of coated granules. Blood sugar was again measured with glucometer after 30 min, 1 h, 2 h and 3 h of glucose administration.

**Statistical Analysis:** Statistical analysis was carried out by one way ANOVA followed by Dunnett's test using Graphpad Prism software. $P<0.05$ was considered significant.

## RESULTS AND DISCUSSION:

**Glucose Tolerance Test (GTT):** GTT is one of the simple ways to find out the hypoglycemic effects of extracts or compounds in animal models. In the present study, GTT was conducted in normal rats. Results for GTT with CWI and its fractions are presented in **Table 1**. Fasting blood sugar level was normal (between 70 to 100 mg/dl) for all the groups. A high dose of glucose increased the sugar level within 30 minutes to more than 1.5 times of FBS as seen in the vehicle control group. The rats pre-treated with CWI showed lower blood sugar levels as compared to the vehicle control group.

Among the three fractions separated from CWI, the CWI-HP showed better hypoglycemic effects than the other two fractions. The blood sugar levels were below 100 mg/dl throughout the study for the CWI-HP group. The difference was highly significant ($p<0.001$) as compared to the vehicle control group. The standard antidiabetic drug glibenclamide also reduced the blood sugar level to the normal range for the whole duration of the study.

**TABLE 1: ORAL GLUCOSE TOLERANCE TEST IN RATS WITH CWI AND COLUMN FRACTIONS**

| Groups | Dose | Blood glucose level (mg/dl) | | | | |
|---|---|---|---|---|---|---|
| | | FBS (0 min) | 30 min | 1 h | 2 h | 3 h |
| Vehicle | 10 ml/kg | 84.67±3.10 | 153.00±1.59 | 134.67±1.28 | 121.67±1.64 | 95.00±2.22 |
| CWI | 75 mg/kg | 90.33±2.01 | 110.33±0.76** | 100.33±1.38** | 101.33±2.13** | 88.00±1.31* |
| CWI-LP | 75 mg/kg | 88.67±2.64 | 133.67±6.45 | 135.00±4.74 | 109.00±0.63 | 106.00±2.55 |
| CWI-MP | 75 mg/kg | 85.33±0.76 | 126.00±4.06* | 126.33±0.91 | 108.33±1.47* | 93.33±1.64 |
| CWI-HP | 75 mg/kg | 88.00±1.67 | 97.67±5.32*** | 97.67±2.01*** | 99.00±3.70*** | 88.00±2.39* |
| Glibenclamide | 1 mg/kg | 86.33±1.38 | 102.67±2.34*** | 94.33±1.64*** | 85.00±1.59*** | 80.33±1.72** |

Data were expressed as mean ± SEM, n=6 rats/group. *Significant from vehicle control at P<0.05. **Significant from vehicle control at P<0.01. ***Highly significant from vehicle control at P<0.001.

Since CWI-HP was found to be the best among the three fractions, a dosage study with CWI-HP was conducted to find the lowest effective dose required to produce a significant difference from the vehicle

control group. The dose range for CWI-HP was 5 to 75 mg/kg in rats and the results are presented in **Table 2**. Among these doses, 25 and 50 mg/kg dosage have shown a highly significant difference from the vehicle control group. The dose of 10 mg/kg was also able to reduce the blood sugar level but was found less effective than the higher dosages.

**TABLE 2: DOSAGE STUDY WITH CWI-HP USING ORAL GLUCOSE TOLERANCE TEST IN RATS**

| Groups | Dose | Blood glucose level (mg/dl) | | | | |
|---|---|---|---|---|---|---|
| | | FBS (0 min) | 30 min | 1 h | 2 h | 3 h |
| Vehicle | 10 ml/kg | 89.00±1.31 | 148.33±2.37 | 135.00±1.93 | 122.00±1.31 | 99.00±2.28 |
| CWI-HP | 5 mg/kg | 83.33±1.47 | 143.00±1.67 | 147.33±6.43 | 121.00±3.84 | 108.00±2.22 |
| CWI-HP | 10 mg/kg | 80.33±5.70 | 109.67±1.72** | 110.67±4.97* | 100.00±1.67* | 81.00±3.16** |
| CWI-HP | 25 mg/kg | 92.33±2.13 | 104.33±0.91** | 105.67±2.48** | 94.00±2.22** | 91.67±1.64* |
| CWI-HP | 50 mg/kg | 86.67±3.10 | 98.33±2.97*** | 103.00±0.96*** | 90.00±2.28*** | 86.67±3.10** |
| CWI-HP | 75 mg/kg | 85.33±3.10 | 94.00±5.97*** | 97.00±4.21*** | 84.67±2.20*** | 83.67±3.87** |

Data were expressed as mean ± SEM, n=6 rats/group. *Significant from vehicle control at P<0.05. **Significant from vehicle control at P<0.01. ***Highly significant from vehicle control at P<0.001.

When active principles present in the extract are unstable in acidic pH, an enteric coating on extract may provide protection of actives in the stomach pH while administered orally. In the present study, the enteric coating was performed on CWI-HP and the isolated molecule CWI-HP-P3 to observe the effect of coating on the stability of actives in the stomach and in turn hypoglycemic activity at a lower dosage than uncoated fraction or isolated molecule. Results on GTT with enteric-coated samples are presented in **Table 3**. While CWI-HP was effective in reducing the blood sugar level at 25 and 50 mg/kg dose, enteric-coated CWI-HP was able to produce a similar hypoglycemic effect at 10 mg/kg dose in rats. In the same line, CWI-HP-P3 showed strong hypoglycemic potential at 10 mg/kg onwards whereas the enteric-coated form of CWI-HP-P3 was able to produce a highly significant reduction in blood glucose level at 5 mg/kg dose in rats.

**TABLE 3: ORAL GLUCOSE TOLERANCE TEST IN RATS WITH ENTERIC COATED CWI-HP AND CWI-HP-P3**

| Groups | Dose | Blood glucose level (mg/dl) | | | | |
|---|---|---|---|---|---|---|
| | | FBS (0 min) | 30 min | 1 h | 2 h | 3 h |
| Vehicle | 10 ml/kg | 90.00±0.96 | 145.33±2.13 | 134.67±1.28 | 117.67±0.91 | 99.67±0.76 |
| Enteric coated CWI-HP | 5 mg/kg | 83.67±2.43 | 114.00±2.03** | 108.33±0.76* | 89.67±2.07*** | 82.33±1.17** |
| Enteric coated CWI-HP | 10 mg/kg | 88.33±1.11 | 106.00±1.82*** | 102.00±1.93** | 94.33±1.28*** | 87.67±0.55* |
| Enteric coated CWI-HP | 20 mg/kg | 90.33±1.64 | 102.67±0.76*** | 98.67±0.55*** | 89.67±2.07*** | 88.33±2.20* |
| CWI-HP-P3 | 5 mg/kg | 89.67±0.76 | 137.67±1.28 | 133.67±4.56 | 116.00±4.21 | 101.00±1.67 |
| CWI-HP-P3 | 10 mg/kg | 84.67±2.20 | 106.33±3.10*** | 109.33±2.74* | 93.00±2.55*** | 84.33±2.20** |
| CWI-HP-P3 | 20 mg/kg | 85.33±2.01 | 101.67±1.80*** | 105.00±1.67** | 96.00±0.73*** | 85.33±1.52** |
| Enteric coated CWI-HP-P3 | 5 mg/kg | 85.00±1.67 | 115.33±1.47*** | 106.67±0.91** | 98.67±0.76*** | 81.33±1.87** |
| Enteric coated CWI-HP-P3 | 10 mg/kg | 84.67±2.64 | 101.00±1.82*** | 96.67±2.56*** | 83.67±2.13*** | 82.67±2.34** |
| Enteric coated CWI-HP-P3 | 20 mg/kg | 87.33±1.17 | 101.00±2.55*** | 100.00±3.59*** | 82.33±5.94*** | 85.33±1.52** |

Data were expressed as mean ± SEM, n=6 rats/group. *Significant from vehicle control at P<0.05. **Significant from vehicle control at P<0.01. ***Highly significant from vehicle control at P<0.001.

The findings in this study clearly indicate the hypoglycemic efficacy of CWI-HP and CWI-HP-P3 in GTT in rats. Moreover, it is also evident that enteric coating on this fraction/molecule provides protection of actives in the stomach and hence very low dose is enough to produce significant hypoglycemic effects.

**Fractionation of CWI:** CWI extract obtained from *Costus pictus* leaves was fractionated by silica gel column chromatography in a flash chromatographic system. The developed method for the fractionation was based on the TLC profile of the extract with different solvent systems. Compounds present in the extract were precisely fractionated into three fractions and designated as CWI-LP (composed of low polar compounds from CWI), CWI-MP (composed of medium polar compounds from CWI) and CWI-HP (composed of high polar compounds from CWI).

CWI and CWI-HP were effective in reducing the blood sugar level and the active fraction CWI-HP was further subjected to the purification of chemical constituents for the identification of the active molecule.

**Isolation and Characterization of Chemical Constituents:** CWI-HP fraction with significant hypoglycemic effects was subjected to column chromatography over silica gel using an isocratic solvent system composed of ethyl acetate and methanol for the purification of active compound/s. The major compound was purified as a white amorphous powder (CWI-HP-P3) and showed a positive Liberman Burchard test with the formation of a violet ring indicating a steroid skeleton. The compound was identified with 1D and 2D NMR analysis and HRMS. The $^{13}C$ NMR spectrum of the compound indicated 35 carbon signals, of which six were for the sugar moiety and 29 were assigned to the aglycone moiety.

Analysis of $^{1}H$ NMR, $^{13}C$ NMR, and DEPT data indicated the molecular formula $C_{35}H_{60}O_6$. This molecular formula was further confirmed by HRMS of the compound at 575.4536 $(M-H)^-$. From a comparison of the $^{1}H$-NMR, $^{13}C$-NMR, HMBC, HSQC and COSY along with HRMS of CWI-HP-P3 and those reported in the literature ascertained the structure of the compound as β-sitosterol-3-O-β-D-glucoside (daucosterol) **Fig. 1** [19]. The compound has already been reported to possess anti-diabetic activity in streptozotocin-induced diabetic rats [20]. β-sitosterol-3-O-β-D-glucoside and it's aglycone sitosterol were reported to increase the fasting plasma insulin levels and improved the oral glucose tolerance in the animal model [21].

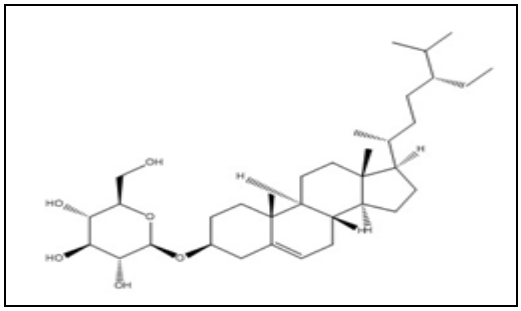

**FIG. 1: STRUCTURE OF β-SITOSTEROL-3-O-β-D-GLUCOSIDE (DAUCOSTEROL)**

**HPTLC Estimation of Active Molecule:** HPTLC estimation of active compound, β-sitosterol-3-O-β-D-glucoside was carried out using a Camag HPTLC system. The HPTLC method as described earlier could precisely estimate the compound present in the water-insoluble extract of *Costus pictus*. Standard compounds in the range 90 pg - 270 pg gave linear response with regression equation y = 11338x – 345.5 for β-sitosterol-3-O-β-D-glucoside. The correlation coefficient ($R^2$) 0.996 indicated a good linear relationship between peak area and concentration of the standard. β-sitosterol-3-O-β-D-glucoside was present in 0.5% w/w in water-insoluble extract *Costus pictus* leaves **Fig. 2**. The HPTLC method presented can successfully separate the active compound present in the extract. The developed HPTLC method is sensitive, selective and reliable and can be used as a tool for routine analysis of the compounds in various samples of *Costus pictus*.

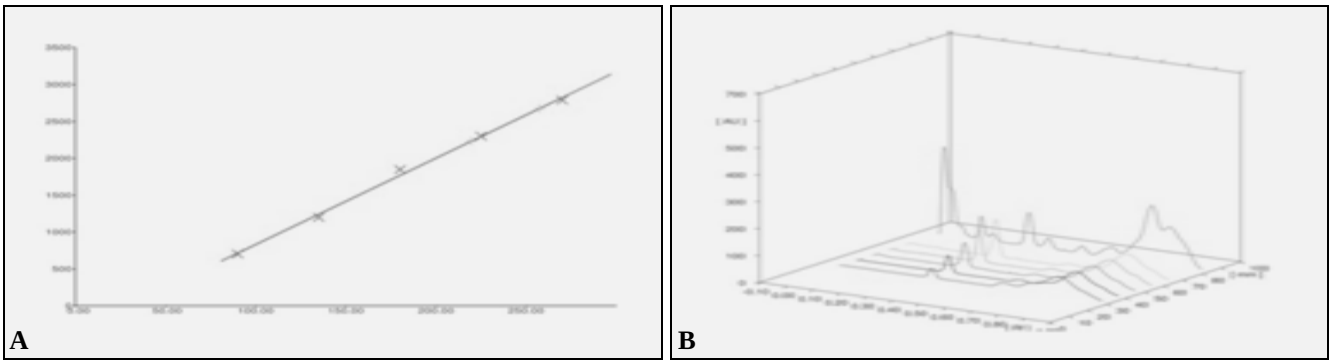


**FIG. 2: HPTLC ESTIMATION OF DAUCOSTEROL: A- CALIBRATION CURVE FOR DAUCOSTEROL; B- HPTLC DENSITOGRAM FOR STANDARD AND CWI**

**CONCLUSION:** Water-insoluble extract (CWI), a high polar fraction (CWI-HP) and isolated compound (β-sitosterol-3-O-β-D-glucoside) of *Costus pictus* leaves showed significant dose-dependent hypoglycemic activity in rats. It can also be concluded that the active fraction/isolated compound was sensitive to low pH of the stomach, and, hence enteric coating would be beneficial to prevent its release in the stomach, facilitating the release directly into the intestine and in turn, reduction in dosage. HPTLC estimation of the active compound revealed the plant as a rich source of β-sitosterol-3-O-β-D-glucoside.

**Compliance with Ethical Standards:** All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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**CONFLICTS OF INTEREST:** The authors declare that there is no conflict of interest.

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