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PHARMACOGNOSTICAL, PHYTOCHEMICAL SCREENING AND INVITRO ANTI-DIABETIC ACTIVITY OF *COSTUS PICTUS* D.DON STEM EXTRACT

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ABSTRACT

This study examines the anti-diabetic properties of ethanolic stem extract from *Costus pictus* D.Don from a pharmacognostic and phytochemical perspective. Physicochemical characterization, quality control, and identification of plant materials are essential for the pharmacognostic assessment. The ethanolic stem extract contains a variety of bioactive compounds. This study also assessed alpha-amylase and alpha-glucosidase enzyme inhibitory effects of the extract on glucose metabolism in vitro. The inhibitory effect of the extract increases with concentration, suggesting natural chemical constituents are responsible for its effectiveness. It has been shown that ethanolic stem extracts of *Costus pictus* D.Don have significant anti-diabetic effects. In addition to bringing valuable insights into *Costus pictus* D.Don's medicinal properties, this research contributes to pharmacognosy and phytochemistry. Diabetes management is possible with the plant's demonstrated anti-diabetic activity, emphasizing its significance as a source of natural compounds. Further exploration of *Costus pictus* D.Don as a potential anti-diabetic drug candidate will be possible.

Key words: *Costus pictus* D.Don, Pharmacognostical characterization, Phytochemical screening, In vitro anti-diabetic activity, Ethanolic stem extract, Alpha-amylase and alpha-glucosidase inhibition.

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INTRODUCTION

Costus pictus D. Don, commonly known as "Insulin Plant" or "Spiral Flag," is a medicinal plant with a rich history in traditional medicine, particularly in the Indian subcontinent (Wallis TE. 1965). Costus pictus, with its distinctive spiral arrangement of leaves and therapeutic potential, has been a focus of pharmacognostical research (Junaid R Shaikh and Patil M K. 2020). This includes the identification, characterization, and assessment of the plant's botanical features, providing a foundation for further exploration. In traditional medicine, *Costus pictus* has been utilized for its anti-diabetic properties (Unuofin, J.O, *et al.* 2018). Diabetes mellitus is a global health concern, affecting millions of individuals worldwide. The prevalence of diabetes is particularly high in India, contributing significantly to the country's disease burden (Apostolidis E, *et al*. 2007, Martinez, M 1996). The need for effective and accessible treatments has prompted researchers to explore traditional remedies, including medicinal plants. Diabetes has reached epidemic proportions globally, and India has not been immune to this health crisis (Rzedowski J, Rzedowski G. 1979). The International Diabetes Federation (IDF) estimates that approximately 537 million people will be living with diabetes by 2035. In India, the prevalence of diabetes is escalating, with diverse factors such as genetic predisposition, lifestyle changes, and dietary habits contributing to its rise. Medicinal plants have long been recognized for their therapeutic potential in managing diabetes (Caceres A, *et al*. 1987). With increasing

awareness of the limitations and side effects of conventional medications, there is a growing interest in exploring natural alternatives. Costus pictus, with its history of traditional use and anecdotal evidence of efficacy, presents an intriguing avenue for research into novel anti-diabetic agents (Argueta V.A, *et al*. 1994). The present study aims to comprehensively explore the pharmacognostical features of *Costus pictus* D.Don, conduct phytochemical screening to identify bioactive compounds, and evaluate the in vitro anti-diabetic activity of its stem extract. The research endeavors to contribute valuable insights into the plant's medicinal properties, providing a scientific basis for its traditional use in diabetes management.

MATERIALS AND METHOD

Collection of Plant material

Stem of the plant *Costus pictus* D.Don was collected from Maduravoyal, Chennai and was authenticated by Dr K.N. Sunil Kumar, Research Officer and H.O.D of Pharmacognosy, Siddha Central Research Institute, Arumbakkam, Chennai.

Pharmacognostical studies

Morphological studies

The fresh stems are studied for its morphological characters like color, odour, taste, stem length, stem width by means of organoleptic test.

Microscopical studies

A careful selection of healthy plants and normal organs was made. A healthy plant's petioles and stems were collected through a cut in the petioles of the plant. Materials was cut into pieces and immersed immediately in a mixture of FAA (formalin (5ml); acetic acid (5ml); 70% ethanol (90ml)). Over 48 hours were spent preserving the sample in fixative FAA. A sharp blade was used to cut specimens into thin transverse sections, which were then stained with safranin. Nikon ECLIPSE E200 trinocular microscope and Zeiss Axio Cam Erc5s digital camera were used for the photography of transverse sections. An indication of magnification was provided by a scale bar.

Powder microscopy

A pinch of the powdered sample was treated with equal volume of phloroglucinol and Conc. HCL and then mounted on a microscopic slide with a drop of glycerol. Characters were observed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss Erc5s digital camera under bright field light. Photomicrographs of diagnostic characters were captured and documented.

Physicochemical studies (Merina B. 2004)

A variety of physicochemical parameters were tested on the powdered stem material, including Ash value, loss on drying, swelling index, foaming index and extractive values.

Phytochemical studies

Extraction

Previously dried, powdered, sieved and stored crude drugs of the stem of *Costus pictus* D.Don was taken. It was extracted by 3 different solvents such as hexane, chloroform and ethanol by means of Soxhlet apparatus. Then the entire extracts were concentrated to dryness using a buchi rotary evaporator under reduced pressure. The final yield of individual extracts was calculated and were stored in labeled sterile bottles under appropriate temperature.

Preliminary phytochemical screening (Comargo M.E.M, *et al*. 2006)

In botanical terms, phytochemicals are chemicals derived from plants and are used to describe a broad range of secondary metabolites. Bioactive compounds are screened for phytochemical activity using phytochemical screening assays, which are a simple, quick, and inexpensive method. The various chemical tests were performed on this hexane, chloroform and ethanol extracts of stem of *Costus pictus* D.Don for the identification of Carbohydrate, Alkaloids, Saponins, Flavonoids, Tannins and Protein by using standard procedure.

In Vitro Anti-Diabetic Assay

Alpha-amylase inhibition assay: (Mabry T.J, *et al.* 1970)

The α-amylase inhibitory activity of the test sample (C.E) was carried out according to the standard method with minor modification (Unuofin et al., 2018). 100 µL of α-amylase solution (0.1 mg/mL) was mixed with different concentrations (10, 20, 40, 80,160 and 320 µg/mL) of test sample, reference standard (Acarbose) and control (without standard/test sample) and pre-incubated at 37 °C for 15 min. Then, 100 µL of starch solution was added to initiate reaction and incubation was done at 37 °C for 60 min., then 10 µL of 1 M HCl and 100 µL of iodine reagent were added to the test tubes. The absorbance of the mixture was measured at 565 nm.

Alpha- Glucosidase Inhibition assay: (Omaye S.T, *et al.* 1979)

The effect of the plant extract (**C.E**) on α-glucosidase activity was determined according to the method described, using α-glucosidase enzyme. The substrate solution p-nitrophenyl glucopyranoside (pNPG) was prepared in 100 mM phosphate buffer, and pH 6.8. 200 µL of α-glucosidase was pre-incubated with different concentrations (10, 20, 40, 80, 160 and 320) of the extracts for 10 min. Then 400 µL of 5.0 mM (pNPG) as a substrate dissolved in 100 mM phosphate buffer (pH 6.8) was then added to start the reaction. The reaction mixture was incubated at 37 °C for 20 min and stopped by adding 1 mL

of Na_2CO_3 (0.1 M). The yellow-colored reaction mixture, 4-nitrophenol, released from pNPG was measured at 405 nm using UV - VIS spectrophotometer. Voglibose was used as a positive control and the inhibitory activity of α-glucosidase was calculated.

RESULTS AND DISCUSSION

Pharmacognostical Studies

The results of the pharmacognostical studies are as follows,

MORPHOLOGY

Colour	: Red & Brown
Odour	: Characteristic
Taste	: Characteristic
Stem length	: 1.2m
Stem width	: 8-13mm

TRANSVERSE SECTION OF STEM

- The stem was long and narrow with wavy margin. Spiraling stems with mottled, striped and spotted patterns.
- Epidermis consists of ground cells and forms the outermost layer. The outermost layer was covered by cuticle and trichomes.
- Ground cells have chloroplast, large vacuoles and they form the mass of stem, leaves and roots
- Phloem is a living vascular tissue responsible for transport of food to all parts of the plant. It contains parenchyma, companion cells, fibres and sieve tubes.
- Xylem transports water and nutrients. It consists of tracheids, vessels, parenchyma and fibres.
- Pith or medulla is a tissue in the stem of vascular plant. It is located in the centre of the stem and it is composed of spongy parenchyma cells.
- Pith consists of thin walled cells which were small and round near vascular bundles and became elongated in the centre of the stem.

Powder Microscopic Observations

The powder preparation includes the following:

Calcium oxalate crystals:

Calcium oxalate is a common biomineral in plants, occurring as crystals of various shapes. It can be found in any tissue or organ in plants and is often formed in the vacuoles of specialized cells called crysal idioblasts.

Tracheid:

A tracheid is a long, lignified cell in the xylem of vascular plants. There are often pits (also known as pupils or guide holes) or decorative on the cell walls of tube cells. When mature, tracheids do not have a protoplast. The main functions are to transport water and inorgqnic salts, and to provide structural support for trees.

Trichomes:

Trichomes obtained from the Greek word "trichoma" meaning "hair", are fine outgrowths or appendages on plants, algae, lichens and certain protists. They are of diverse structure and function. Examples are hairs, glandular hairs, scales and papillae.

Fibres:

Fibres are greatly elongated cells whose long, tapering ends interlock, thus providing maximum support to a plant. They often occur in bundles or strands and can be found almost anywhere in the plant body, including the stem, root and leaves.

Physiochemical Studies

Table 1: The physiochemical constant analysis of stem of *Costus pictus* D. Don

SI.NO	PHYSIOCHEMICAL CONSTANT	PERCENTAGE (% W/W)
1	Ash values	
	Total ash	29±1.0
	Water soluble ash	7±0.34
	Acid insoluble ash	9±0.98
	Sulphated ash	15.5 ±0.62
2	Extractive values	
	Alcohol soluble extractive value	22±0.52
	Water soluble extractive value	5±0.4
3	Loss on drying	47.5±1.5
4	Foaming index	Less than 100
5	Swelling index	20ml

Phytochemical Studies:

The result of phytochemical studies are as follows

Table 2: Percentage yield of successive extract of stem of *Costus pictus* D.Don

Si. No	Extract	Method of extraction	Physical nature	Colour	Yield (%w/w)
1	Hexane	Soxhlet extraction	Semi solid	Blackish green	3.42%
2	Chloroform		Semi sticky	Green	2.64%
3	Ethanol		Semi solid	Cherry red	6.21%

Table 3: Preliminary phytochemical screening on the stem of *Costus pictus* D.Don

SI.NO	PHYTO CONSTITUENTS	POWDER	HEXANE	CHLOROFORM	ETHANOL
1	Carbohydrates	+	+	+	+
2	Alkaloids	+	+	+	+
3	Steroids and sterols	+	+	+	+
4	Glycosides	-	-	-	-
5	Saponins	+	+	+	+
6	Flavonoids	+	-	-	+
7	Tannin/phenolic	+	+	+	+
8	Fixed oil	+	+	+	+

[+] POSTIVE [-] NEGATIVE

Table: 3 Alpha-amylase inhibition assay

	Control	0.041	0.046	0.044						
Acarbose	Conc. (µg/mL)	Singlet	Duplicate	Triplicate	Singlet	Duplicate	Triplicate	Mean	SD	IC50
	10	0.084	0.089	0.085	51.1904762	48.31461	48.23529	**49.24679**	1.6837466	
	20	0.221	0.224	0.228	81.4479638	79.46429	80.70175	**80.538**	1.0019261	
	40	0.403	0.406	0.405	89.8263027	88.66995	89.1358	**89.21069**	0.5818016	**3.29**
	80	0.745	0.747	0.742	94.4966443	93.84203	94.07008	**94.13625**	0.3322838	
	160	1.186	1.183	1.187	96.5430017	96.11158	96.29318	**96.31592**	0.2166078	
	320	1.914	1.918	1.913	97.8578892	97.60167	97.69995	**97.71984**	0.1292629	
C.E	Conc. (µg/mL)	Singlet	Duplicate	Triplicate	Singlet	Duplicate	Triplicate	Mean	SD	
	10	0.053	0.058	0.055	22.6415094	20.68966	20	**21.11039**	1.3700931	
	20	0.06	0.064	0.063	31.6666667	28.125	30.15873	**29.98347**	1.7773263	
	40	0.081	0.085	0.083	49.382716	45.88235	46.98795	**47.41767**	1.7893102	**34.66**
	80	0.263	0.264	0.267	84.4106464	82.57576	83.5206	**83.50233**	0.9175808	
	160	0.856	0.859	0.858	95.2102804	94.64494	94.87179	**94.909**	0.284503	
	320	1.454	1.453	1.456	97.1801926	96.83414	96.97802	**96.99745**	0.1738443	

Conc.(µg)	C.E	Acarbose
10	21.110388	49.246792
20	29.983466	80.538001
40	47.417674	89.210685
80	83.502334	94.136253
160	94.909004	96.315919
320	96.99745	97.719835

Table: 4 Alpha- Glucosidase Inhibition assay

				Mean							
Control	0.632	0.638	0.635	0.635							
	Conc.(µg/mL)	Singlet	Duplicate	Triplicate		Singlet	Duplicate	Triplicate	Mean	SD	IC50
VOGLIBOSE	10	0.485	0.488	0.481		23.62205	23.149606	24.25197	**23.674541**	0.5530527	
	20	0.231	0.236	0.239		63.62205	62.834646	62.3622	**62.939633**	0.6364491	
	40	0.169	0.161	0.166		73.38583	74.645669	73.85827	**73.963255**	0.6364491	**17.23**
	80	0.084	0.088	0.082		86.77165	86.141732	87.08661	**86.666667**	0.4811103	
	160	0.064	0.066	0.069		89.92126	89.606299	89.13386	**89.553806**	0.3963168	
	320	0.038	0.032	0.035		94.01575	94.96063	94.48819	**94.488189**	0.4724409	
C.E	Conc.(µg/mL)	Singlet	Duplicate	Triplicate		Singlet	Duplicate	Triplicate	Mean	SD	
	10	0.524	0.522	0.528		17.48031	17.795276	16.85039	**17.375328**	0.4811103	
	20	0.358	0.352	0.355		43.62205	44.566929	44.09449	**44.094488**	0.4724409	
	40	0.265	0.269	0.263		58.26772	57.637795	58.58268	**58.16273**	0.4811103	**36.04**
	80	0.205	0.203	0.207		67.71654	68.031496	67.40157	**67.716535**	0.3149606	
	160	0.139	0.133	0.137		78.11024	79.055118	78.4252	**78.530184**	0.4811103	
	320	0.106	0.102	0.104		83.30709	83.937008	83.62205	**83.622047**	0.3149606	

Conc.(µg/mL)	VOGLIBOSE	C.E
10	23.67454068	17.375328
20	62.93963255	44.094488
40	73.96325459	58.16273
80	86.66666667	67.716535
160	89.55380577	78.530184
320	94.48818898	83.622047

Figure 1a: T.S OF STEM A SECTOR ENLARGED 10X

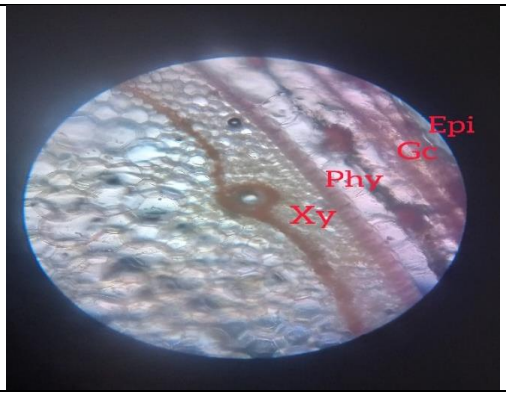


Figure 1b; T.S OF STEM CENTRE PART VIEW 10X (Epi – Epidermis, GC – Ground cells, Phy – Phloem, Xy – Xylem, Pi – Pith)

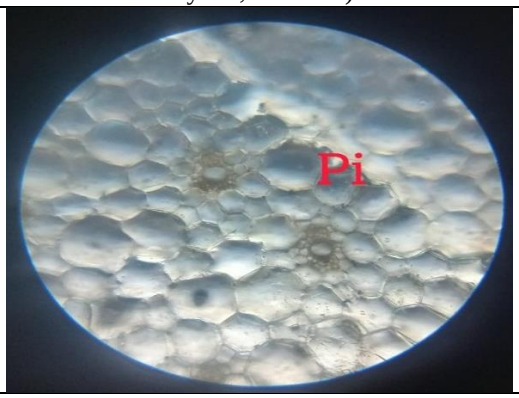


Figure 1c: CALCIUM OXALATE CRYSTAL

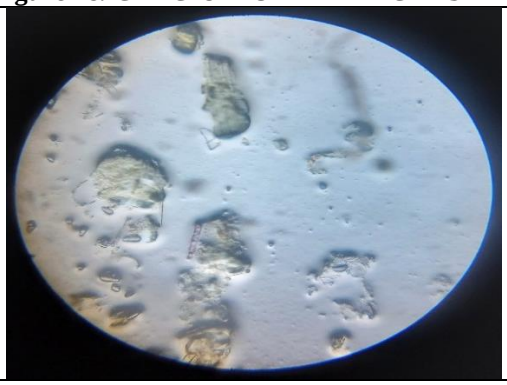


Figure 1d: TRACHEID

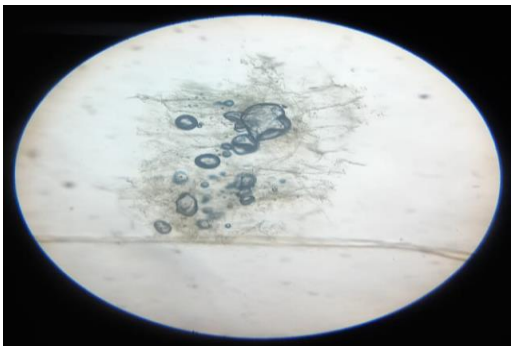


Figure 1e: TRICHOMES

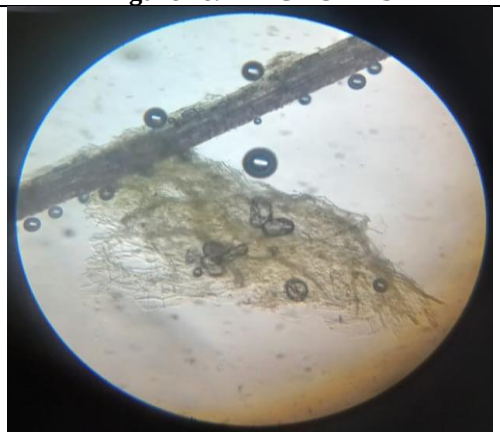
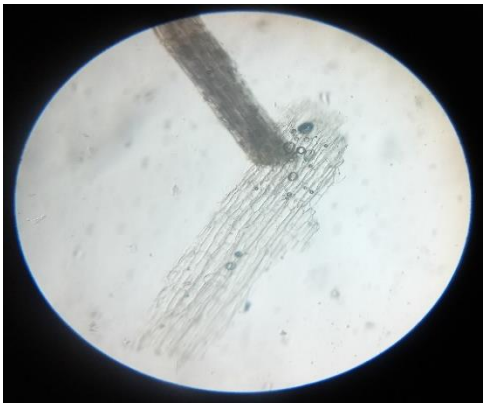


Figure 1f:FIBRES



ANTI DIABETIC ACTIVITY:

Alpha-amylase inhibition assay:

The IC50 value of the given test sample (C.E) and the reference standard (Acarbose) was found to be 34.66 µg/mL and 3.29µg/mL, respectively.

Figure 2a: STANDARD:

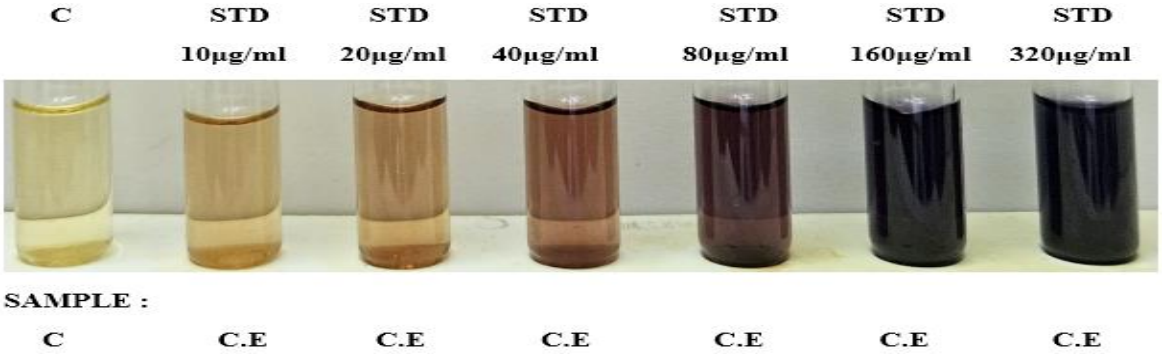
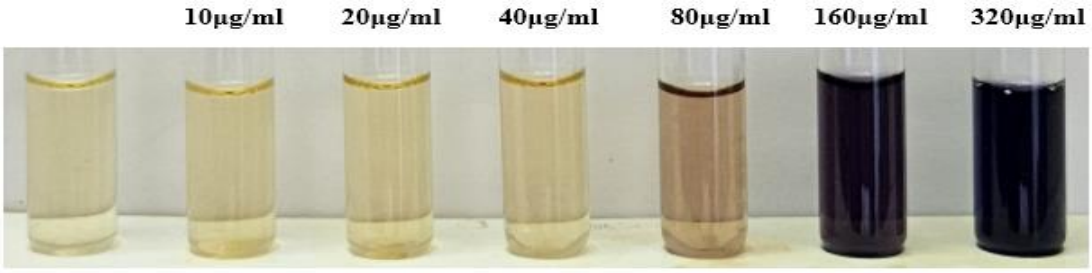


Figure 2b SAMPLE :



Alpha- Glucosidase Inhibition assay:

The IC_{50} value of the given sample (**C.E**) and the standard drug (**Voglibose**) was found to be **36.04 µg/mL** and **17.23 µg/mL**

Figure 3a: STANDARD:

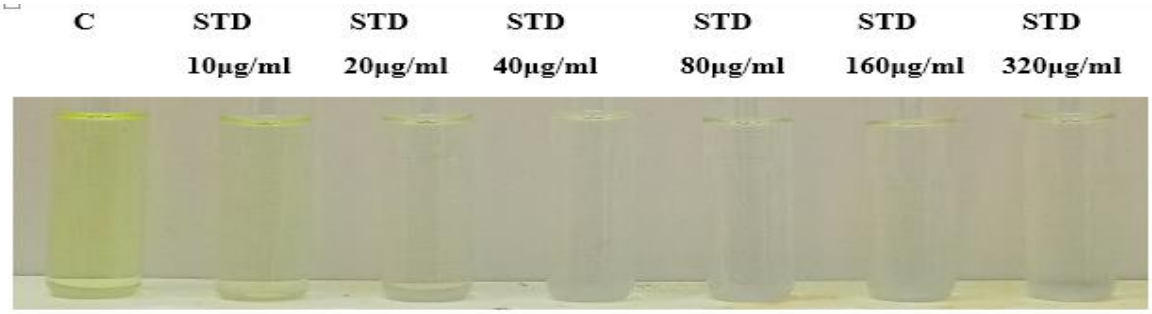


Figure 3b: SAMPLE :

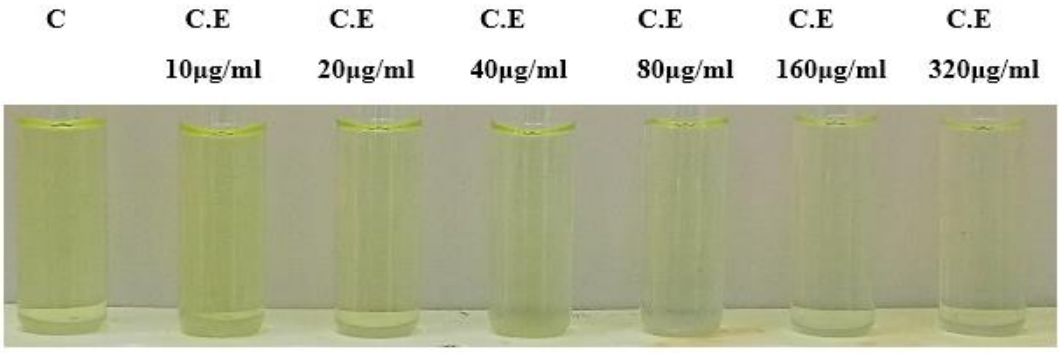


Figure 4:

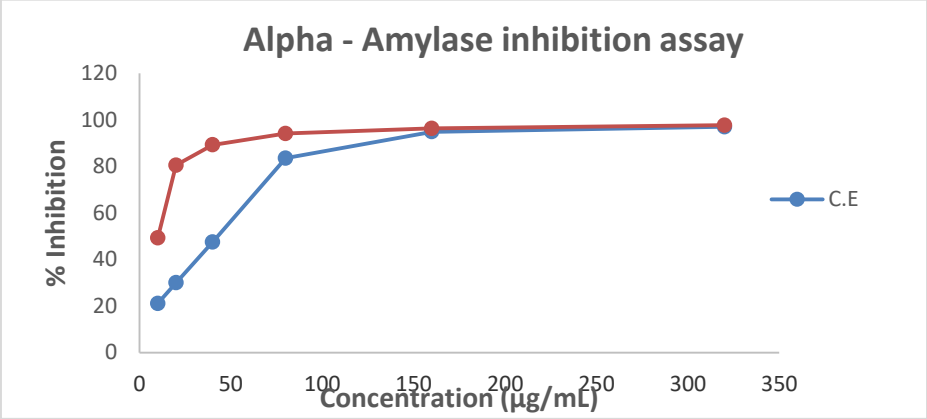
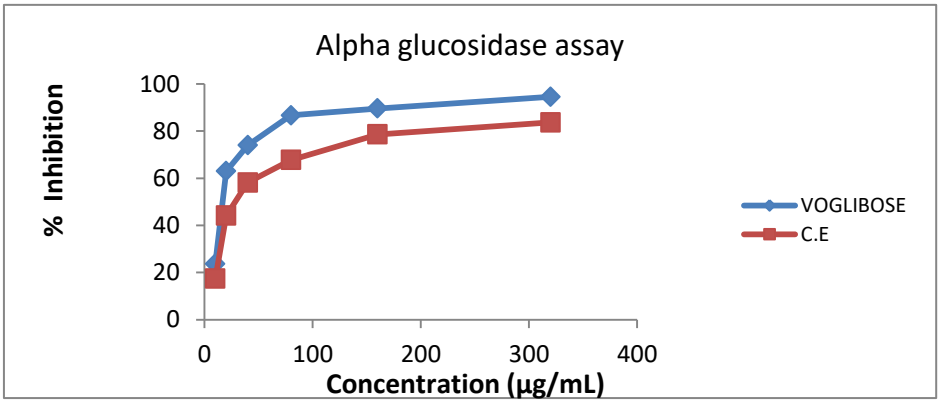


Figure 5



CONCLUSION

The ethanolic extract derived from the stems of *Costus pictus* D.Don demonstrated notable inhibition percentages for both alpha-amylase and alpha-glucosidase enzymes. The inhibitory effects exhibited a positive correlation with increasing extract concentration. The presence of natural chemical constituents in the ethanolic stem extract of *Costus pictus* D.Don proved effective in inhibiting alpha-amylase and alpha-glucosidase. In conclusion, this study establishes that the ethanolic stem extract of *Costus pictus* D.Don showcases promising anti-diabetic activity

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