Moroccan Journal of Chemistry
ISSN : 2351-812X

Mor. J. Chem., 2023, Volume 11, Issue 4, Page 917-931
https://revues.imist.ma/index.php/morjchem
https://doi.org/10.48317/IMIST.PRSM/morjchem-v11i04.39992

Anti-Hyperglycaemic properties of an ethanolic extract from *Costus spicatus* (jacq) on Streptozotocin-induced diabetic male albino Wistar rats

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Received *05 May 2023,*
Revised *19 July 2023,*
Accepted *21 July 2023*

Citation*: Sivalingam A. M., Altemimi A.B., Abdul-Abbas S.J., Pandian A., Lakshmanan G., Parasuraman G., Nisha B., Dutta R., Najim M.A.A., Cacciola F., Devaraj N. (2023) Production of Hybrid Biochar by Retort-Heating of Elephant Grass for Waste Management and Product Development, Mor. J. Chem., 11(4), 917-931*

Abstract: In this contribution, for the first time an ethanolic extract of *Costus spicatus*, was screened for evaluating its composition followed by its *in vitro* antidiabetic activity. Both spectrometric and gas chromatography techniques were employed along with the antidiabetic activity on (STZ)-Streptozotocin-induced male albino Wistar rats. A rich phytochemical content, made up of tannins, saponins, flavonoids, steroids, etc, was attained together with a high amount of ash content (11.3%) and moisture content (1.27%); further, a total of 25 compounds were positively identified by GC-MS analysis. For antidiabetic activity, different treatment period of 0th day, 21th days, 45th days were taken into consideration. Histopathological studies, considering high blood glucose levels, Serum Glutamic oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), and Alkaline Phosphatase (ALP) levels highlighted no damage in the pancreas tissue cells. Based on the findings of this study, such an insulin plant might be considered a potential source and therapeutic agent for diabetic patients.

Keywords: *Costus spicatus;* GC-MS; Histopathological studies; albino Wistar rats.

1. Introduction

Diabetes mellitus is a premier way of life sickness and is the most difficult general medical condition of the 21st century with an overall pervasiveness through the 387 million (8.3%) anticipated 592 million by 2035 (Thiruvengadam & Peter, 2022; Jeon *et al.,* 2023). The individuals (77%) through the diabetes get by in low and center pay nations. Because of its expanded predominance, diabetes accomplished worldwide consideration as it fundamentally influences human wellbeing (Roglic, 2016). Internationally, it is assessed that 425 million in individuals (8.8%) grown-up in 20-79 years are living with diabetes mellitus disease (Salleh *et al.,* 2021). At first, the body makes more insulin to hold glucose levels within proper limits; however, the pancreas at that point wears out and blood

glucose levels become hazardously high (Artasensi *et al.,* 2020). Antidiabetic agents from medicinal plants act as insulin mimickers or insulin secretagogues, and traditional medicine from readily available herbs offers high potentials for the discovery of new antidiabetic drugs (Zhang *et al.,* 2020; Ouahabi *et al.,* 2023). Isolation of secondary metabolites from plants and its anti-hyperglycaemic activity has a major impact on the treatment of diabetes. Some of the classes of active metabolites which has been reported to exhibit anti-hyperglycemic activity are flavonoids, alkaloids, phenolics and terpenoids (Sun *et al.,* 2002; Loukili *et al.,* 2022; Suryelita *et al.,* 2023; Ragadhita *et al.,* 2023). These secondary metabolites or the combination of plant extracts might exert their synergetic effect in the management of diabetes. Flavonoids isolated from *Hyphaene thebaica* exhibited a significant antidiabetic activity (Pinaffi *et al.,* 2020). Antidiabetic activity medicinal plants having lesser secondary issues acting as insulin mimickers or insulin secretagogues offers high possibilities for the disclosure of new enemy of diabetics (Jacob *et al.,* 2002). The Herbal Medicine Scenario remains one of the worlds is India and twelve driving biodiversity and communities. The therapeutic utilization of plants appears to have been created through perception of creatures and experimentation (Zhu *et al.,* 2020). *Costus* is customarily utilized as a restorative spice for the most part for its tonic, energizer, carminative, diuretic, stomach related and germicide properties. It is additionally utilized for restoring oedema, wheezing (dyspnoea), hemorrhoids and spermaturia. In the Siddha medication framework, C. igneus root has been utilized as powder (chooranam), decoction (kudineer) and oil (thylam). It has been accounted to contain retinoids and alkaloids named saussure, inulin, and gum (Zhao *et al.,* 2020). Conventional medications got mostly from plants assume a significant part in the administration of diabetes mellitus.

In the Streptozotocin model, the pancreatic β-cells can be harmed or annihilated with the assistance of streptozotocin (Shwetha *et al.,* 2020). After the administration of an antidiabetic drug, the islets of Langerhans containing beta cells are supposed to be re-established to almost ordinary in streptozotocin-actuated diabetes in rodents (Colombo *et al.,* 2020). The perceptions like basal vacuolization, hypertrophy in glomeruli, glycogen amassing, degeneration of cylindrical epithelium, glomerulosclerosis, expanded mesangial framework, hyalinization, thickening of layer, and so forth might be noted in the histopathology of the kidney (Vijayakumar *et al.,* 2020). The slight improvement in the pancreas, kidney, liver, lungs and cerebrum tissue might be because of the cell reinforcement properties of the antidiabetic medication and its ability to search the free extremists created from streptozotocin, aside from its job in upgrading the practical capacities of the resistant framework (Badeggi *et al.,* 2020). In the kidney, hypertrophy is seen in both the glomerular storm cellar layer and vessels that might add to finishing stage renal harm. Streptozotocin (STZ) is diabetogenic in light of the fact that it specifically obliterates the insulin-delivering beta cells by initiating rot. *C. spicatus* rhizome has long been used as a folk medicine for the management of diabetes determined to be effective of inhibition using α-amylase activity. Thus, α-amylase activity can decrease carbohydrate digestion and reduce postprandial hyperglycemia (Govindaraju *et al.,* 2002).

The objective of present work was to explore for the first time the in vivo anti-diabetic capabilities of an herbal medicinal plant, *Costus spicatus* ethanolic extract on antidiabetic albino Wistar rats.

2. Methodology

2.1. Chemicals and reagents

The chemicals were purchased from Himedia limited, Sisco Research limited, India. chemicals are Streptozotocin (STZ), H&E stain, DPX, ethanol, Ethylene Diamine Tetra Acetic Acid (EDTA), methanol, Glibenclamide, India.

2.2 Sample collection

The medicinal plant *Costus spicatus* was collected from Saliyamangalam (Post) Thanjavur (Dt), Tamil Nadu (Figure 1). The plant identified and herbarium deposited at St. Josephs College, Trichy voucher specimen No SAM 001, authenticated by Rev Dr. S. John Britto SJ, Director, The Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College (Autonomous), Tiruchirappalli, Tamilnadu, India (Azhagumadhavan *et al.*, 2017).

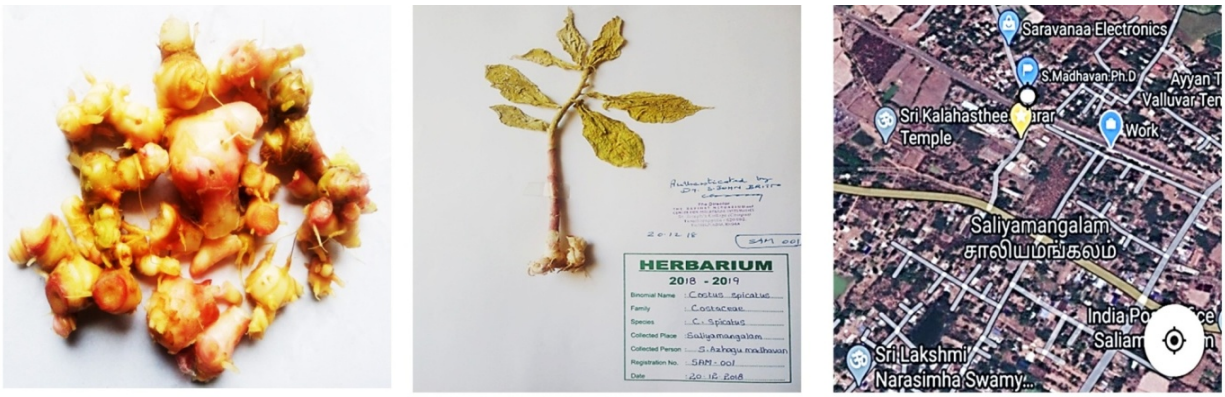

Figure 1. Collection and authentication of plant. (a) Rhizome part of the *Costus spicatus*, (b) Plant identified and herbarium deposited. (c) Plant collected location

2.3 Extraction, Phytochemical and physico-chemical analysis

Collected plant materials washed with distilled water, shade dried at room temperature, and then grinded made into fine powder. The plant extract was prepared using methanol, ethanol, and water (70%) using a solvent extractor. The solvent extractor (DIONEX) was used at conditions 15 atm and 20 °C (Khandalwal *et al.,* 2008). The dried material was kept in a refrigerator. In this study total ash, moister content and extractive values was determined by placing in a crucible and porcelain crucibles weighted (Azhagumadhavan *et al.,* 2017).

2.4. Determination of fluorescence behaviour

The plant powders kept in fluorescence observed colour intensity in short (254 nm) and long UV (365 nm). Fluorescence behaviour used to confirm highest sensitivity of colour variations. The rhizome powder sample was treated with different synthetic reagents (Smina *et al.,* 2020).

2.5. Gas Chromatography Mass Spectrometry (GC-MS) analysis

GC-MS analyses were performed using an Agilent gas chromatograph (Agilent HP-6890). A split method (10:1) was used (Yang *et al.,* 2020). Interfaced mass spectrometer (GC-MS) equipped with a straight deactivated 2 mm direct injector liner and a 15 m All tech EC- column (50 µL–D, 0.5 µL film thickness); oven temperature starts at 35°C, hold, thane ramp at 20°C/min to 300°C and holding for 5 min. The helium carrier gas was set at 2 mL/min. A JEOL GC mate II bench top double focusing magnetic sector mass spectrometer operating in electron ionizing (EI) mode with TSS20, 001 software was used for all analysis. Low – resolution mass spectra were acquired at a resolving power of 1000 (20% height defection) and scanning from *m/z* 25 to *m/z* 700 at 0.3 s per scan with a 0.2 s inter–scan delay. High resolution mass spectra were acquired at a resolving power of 5000 (20% height defection and scanning the magnet from m/z 65 to m/z 750 at 1s per scan. National Institute Standard and Technology (NIST) having more than 62,000 patterns, identification of the unknown components was

carried out by matching their recorded spectra with the data bank mass spectra of NIST library V 11 provided by the instrument software (Yang *et al.*, 2020).

2.6. Animal maintenance

Albino Wistar male rats, 10 weeks old, with a body weight (bw) ranging from 180–220 g, maintained at 24±2°C with the humidity (30-70%) were employed. The light period was maintained for 12/12 hours, light/dark. All animals were fed a standard laboratory control diet and provided with tap water ad libitum. Animal experiments were performed by following the guideless provided by CPCSEA and IAEC (Reg. No: 685/PO/Re/S/2002; dated 21st August 2002 and KMCRET/Ph.D/22/2018-19). The animals induced (IP-Intra peritoneal injection of STZ – dissolved 0.1 M cold sodium citrate buffer, pH-4.5, at a dose conditions 55 mg/kg -IP), glucose solution (5%) and during night induced hypoglycaemia. Blood glucose level range 250 mg/d1, diabetic experiment wistar Albino rat's based on dose, it was selected, per-formed based on previous literature (Azhagumadhavan *et al.,* 2018).

2.7. Blood glucose measurement

Before the blood glucose analysis, the rats were deprived of the food overnight, tail amputation was performed to collect the blood in the early morning for fasting blood glucose (FBG) on intervals of 0, 21 and 45 d. The following models of glucometers which were commonly used in clinical practice were used for the measurements: ACCU-CHECK Compact Plus.

2.8. Pancreas isolation and fixation

To carry out the isolation and fixation of pancreas, the rats were euthanized with chloroform; blood was collected from the left ventricle followed by isolation and preservation of pancreas and small intestine sections in formalin (10%) to perform the histological studies. One of the liver lobes and left kidney were absorbed in the normal saline and used for antioxidant ability of assay.

2.9. Pancreas histological assessment and determination of cross section area

In the histological assessment, pancreas was fixed with buffered formalin (10%) and an automatic tissue processor (Leica TP 1020, Germany) was used for processing. The processed tissues were inserted in paraffin. Then section of the pancreas (10 µm) was sectioned rotating microtome was used for sectioning of the tissue blocks. Later, de-paraffinization was performed then stained process with 0.1% Haematoxylin and Eosin followed by colour observed. The histological sections were examined in a under the microscope at 40 x magnification (Olympus CK40-F200, Japan). Each of the pancreatic pieces was observed in an eyepiece camera (Amscope MD35). Adobe photoshop C6 was used to determine the intersections of the pancreatic islets. Point counting and Calalieri method were used for determining the cross-sectional area (Al-Rowaily *et al.*, 2020; Altunkaynak *et al.*, 2009).

2.10. Experimental Design and Collection of sample

The Wister albino male rats formed six groups; in each group the rats injected with citrate buffer solution group 1; normal, group 2nd diabetic control, group 3rd diabetic+Glibenclamide (5 mg/kg) standard, treatments (4 weeks), group 4 diabetic+rhizome extract (300 mg/kg), 5th diabetic+rhizomes extract (500 mg/kg), all experimental animals ketamine chloride (24 mg/kg) were used, overnight blood collected without EDTA. Afterwards, separated plasma serum, centrifuged focused biochemical estimation. At the end of the experimental phase (day 45), the animals were deprived of food and sacrificed. For the blood glucose assessment, blood was collected in a tube containing EDTA; and for insulin level measurement, the plasma was isolated from the blood by centrifugation. Pancreas and

skeleton muscles were dissected out washed in ice-cold saline and stored in 10% formalin for further studies.

2.11. Statistical analysis

Each experiments records point has been carried out a minimal of three times. To exhibit trial changeability, information had been communicated everyday with touching on well-known deviation. Two-followed mixed Student's t-tests were carried out to set up the factual that means of contrasts between trial checks comparing control. The significant of examinations factual importance, a worth of $p < 0.05$ was viewed huge. Diagrams have been evaluated utilising Graph Pad Prism 9.0.1 version Software) (Arjun *et al.*, 2017).

3. Results and Discussion

3.1. Phytochemical Analysis

The phytochemical analysis of *C. spicatus* was carried out with different solvents (water, ethanol and methanol) extracts used (Table 1). Saponins, steroids and polyphenolics were present in the methanol extract apart from tannins and flavonoids there were absent; in the ethanol extracts all these bioactive compounds were present. In the water extract tannins and saponins were absent, differently from steroids, flavonoids and polyphenols that were present (Boye *et al.*, 2020).

Table 1. Secondary metabolites of qualitative analysis of different solvents in rhizome extract of *Costus spicatus*

S.NO	Secondary Metabolites	Water	Ethanol	Methanol
1.	Tannins	-	+	-
2.	Saponins	-	+	+
3.	Flavonoids	+	+	-
4.	Steroids	+	+	+
5.	Polyphenols	+	++	+

(+) indicates good intensity and (-) sign indicates low intensity

3.2. Physicochemical Parameters

Various physicochemical parameters such as total ash content, water soluble content, sulphate and acid insoluble ash contents (Table 2), along with foreign organic matter, swelling index, moisture content and foaming index were analyzed (Table 3) (Bayrami *et al.*, 2019; Madhavan *et al.*, 2021). The extraction efficiency in various solvents is presented in Table 4. The chemical analysis of a plant extract is crucial for the detection of adulteration or improper handling of medications (Abubakar *et al.*, 2020). At the present investigation of *C. spicatus* has characteristic anatomy and proximate analysis was used to distinguish it from other members of Costaceae family (Karimi *et al.*, 2020).

Table 2. Ash values of the *Costus spicatus* leaves.

S. No.	Parameters	Values
1	Total Ash	11.3%
2	Acid Insoluble Ash	2.5%
3	Water Soluble Ash	7.0%
4	Sulphated Ash	9.5%

Table 3. Moisture content, foreign organic matter, foaming index and swelling index for *Costus spicatus* extract

S.NO	Parameters	Values
1	Moisture Content	1.27%
2	Foreign Organic Matter	Nil
3	Foaming Index	Less than 100
4	Swelling Index	0.3 cm

Table 4. Extractive values *Costus spicatus* extract

S. No.	Solvent	Values %(w/w)
1	Ethanol	11
2	Methanol	7
3	Water	4
4	Chloroform	2
5	Ethyl Acetate	1
6	Petroleum Ether	5

3.3. Fluorescence behaviour

The fluorescent quality of the plant extract was evaluated under UV light at 254 and 365 nm. The plant powder exhibited at visible light a brown colour, a black one at short UV 254 nm and dark black at long UV 365 nm (Table 5). The outcomes got from the current fluorescent examinations help to check any debasements present in rhizome powder of *C. spicatus*. Some phytochemical factors can be found in visible light (Figure 2A), and different compounds colour variation can be observed in UV (Figure 2B,C). A no fluorescent compound might be fluorescent in case it is blended in with contaminations as previously reported (Abubakar *et al.*, 2020; Madhavan *et al.*, 2020).

Table 5. Fluorescence of *Costus spicatus* extract

S.No	Analysed phytochemical factor	Visible Light	Short UV 254 nm	Long UV 365 nm
1	Plant powder (pp)	Light Brown	Black	Dark Black
2	PP with Water	Light Brown	Light Brown	Dark Brown
3	PP with Hexane	Light Brown	Dark Brown	Brown
4	PP with Chloroform	Light Brown	Creamish white	Yellow
5	PP with Methanol	Light Brown	Yellow	Dark Black
6	PP with Acetone	Brown	Dark Black	Brown
7	PP with IN Sodium hydroxide in water	Light Brown	Brownish -Yellow	Light Yellow
8	PP with IN Hydrochloric acid	Dark Brown	Off White	Light Brown
9	PP with Sulphuric acid with an equal amount of water	Light Black	Dark Brown	Light Black
10	PP with Nitric acid diluted with an equal amount of water	Dark Yellow	Light Brown	Brown

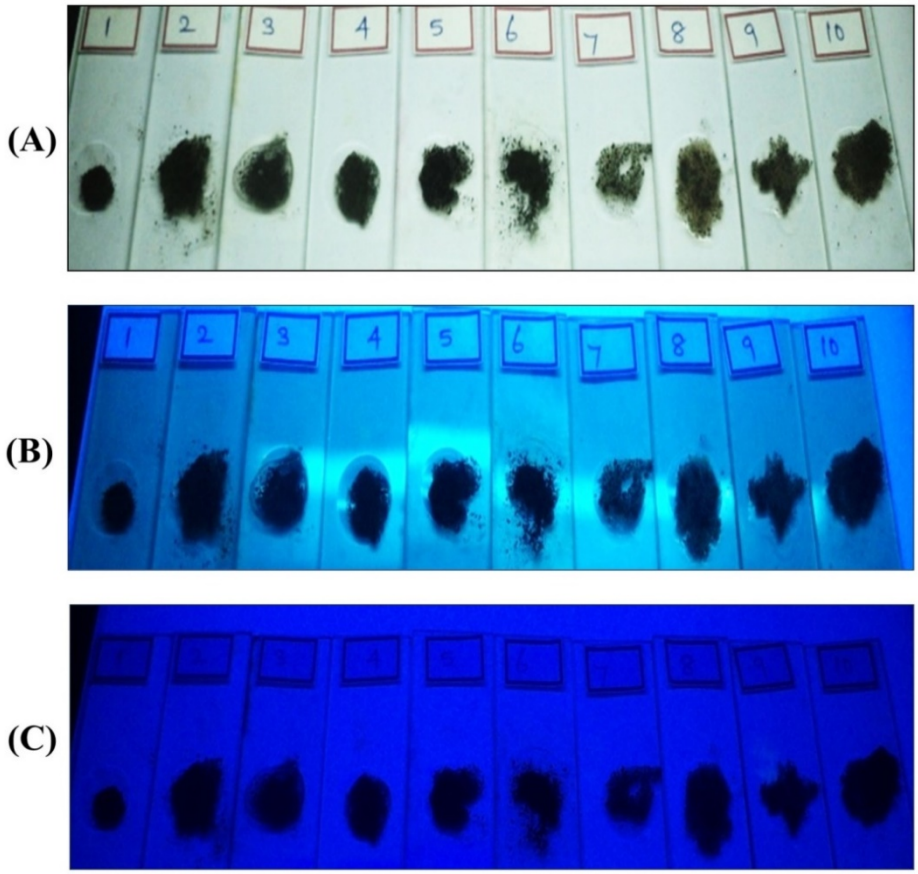

Figure 2. Fluorescence characteristics of crude powdered drugs and emission various colour radiations. (a) Visible light, (b) Short UV at 245 nm, and (c) Long UV at 365 nm.

3.4. GC-MS Analysis

The GC-MS analysis highlighted in *C. spicatus* the presence of 25 major bioactive compounds (Table 6), of whose several biological activities have been reported e.g. antimicrobial, antioxidant, anti-inflammatory, etc (Lee-Rangel *et al.*, 2022; Rahamouz-Haghighi *et al.*, 2022; Vanpure *et al.*, 2022; Kasim *et al.*, 2022; Padma *et al.,* 2019; Molla *et al.*, 2022; Mukkamula *et al.*, 2022; Geetha *et al.*, 2019; Guguloth *et al.*, 2022; Quranayati *et al.*, 2022; Saravanan *et al.*, 2022; López-Cabeza *et al.*, 2022; Dawwam *et al.*, 2022; Ahmed *et al.*, 2022; Singh *et al.*, 2022; Abdel-Motaal *et al.*, 2022; Madhavan *et al.*, 2019).

3.5. Histopathology

Results of anti-diabetic histopathology results of albino Wister rats are shown in Table 7. Each animal group was administrated with *C. spicatus* rhizome extract; at a dose of 300 mg/kg rats some changes in pancreas were noticed and for instance for group IVth body weight ranged from 192±2.79# (0 day) to 181.66±2.23** (21st day) and to 182.51±1.47 (45th day).In terms of plasma glucose levels in normal and experimental rats, at a dose of 300 mg/kg rats for group IVth ranged from 267.73±0.75# (0 day) to 101.5±1.49** (21st day) and to 95.17±0.11**(45th day) (Table 8). Some of features such as arrangement of hepatic cords, nucleus development and sinusoidal spaces appear normal on histopathological examination. Diabetic+plant *C. spicatus* rhizome extract administrated continuously at a dose of 300 mg/kg ranged for SGOPT (IU/L) from 0 day 124.89±54.7** to 45th day 127.87±0.09. The Wistar albino rats group of pancreas diabetic+plant *C. spicatus* rhizome extract by a dose of 500 mg/kg rats change pancreas 0 d 106±2.19** and 45 d 73.67±2.37$^{\#}$ diabetic control (Table 9; Figure 3).

Table 6. Compounds identified with GC-MS and their biological activity with applications.

No.	Compounds	Molecular weight g/mol	Molecular Formula	Retention time	Activity and applications
1	Cyclohexasiloxane, Dodecamethyl-	444	$C_{12}H_{36}O_6Si_6$	9.97	Anti-seizure (Madhavan *et al.*, 2020)
2	Cycloheptasiloxane, Tetradecamethyl-	444	$C_{12}H_{36}O_6Si_6$	11.95	Antioxidant, antihyperglycemic (Lee-Rangel *et al.*, 2022)
3	Benzoic Acid, 2,4-Bis(Trimethylsiloxy)-, Trimethylsilyl Ester	370	$C_{16}H_{30}O_4Si_3$	13.92	Antioxidant (Rahamouz-Haghighi et al., 2022)
4	Cyclononasiloxane, Octadecamethyl	592	$C_{16}H_{48}O_8Si_8$	15.62	Antimicrobial, anti-inflammatory (Vanpure *et al.*, 2022)
5	Benzoic Acid, 2,6-Bis(Trimethylsiloxy)-, Trimethylsilyl Ester	370	$C_{16}H_{30}O_4Si_3$	17.04	Antimicrobial and anti-inflammatory (Kasim *et al.*, 2022)
6	Batilol	344	$C_{21}H_{44}O_3$	20.73	Antioxidant, anti-hyperlipidemia (Padma *et al.*, 2019)
7	Tetracosamethyl-Cyclododecasiloxane	888	$C_{24}H_{72}O_{12}Si_{12}$	22.19	Antifungal, antimicrobial, antibacterial (Molla *et al.*, 2022)
8	1-Heptacosanol	396	$C_{27}H_{56}O$	22.27	Antioxidant, antimicrobial and anti-diarrheal activity (Mukkamula *et al.*, 2022)
9	1-Octadecanol, 18-Bromo-	348	$C_{18}H_{37}BrO$	22.31	Antimicrobial (Mukkamula *et al.*, 2022)
10	Valtrate	422	$C_{22}H_{30}O_8$	22.37	Antibacterial (Mukkamula *et al.*, 2022)
11	6-(4-Adamantan-1-Yl-Piperazin-1-Yl)-N-[4-(Chloro-Difluoro-Methoxy)-Phenyl]-N',N'-Dimethyl-[1,3,5]Triazine-2,4-Diamine	422	$C_{22}H_{30}O_8$	22.42	Antibacterial, antioxidant, anti-inflammatory, anticancer, diuretic (Geetha *et al.*, 2019)
12	14-.Beta.-H-Pregnane	533	$C_{26}H_{34}ClF_2N_7O$	22.73	Antioxidant, anti-inflammatory (Guguloth *et al.*, 2022)
13	Tetrapentacontane, 1,54-Dibromo-	288	$C_{21}H_{36}$	22.89	Antioxidant (Guguloth *et al.*, 2022)
14	Cyclononasiloxane, Octadecamethyl-	914	$C_{54}H_{108}Br_2$	23.01	Chemopreventive (Guguloth *et al.*, 2022)
15	Cyclododecasiloxane, Tetracosamethyl-	666	$C_{18}H_{54}O_9Si_9$	23.15	Reductase inhibitor, antioxidant (Molla *et al.*, 2022)
16	4h-1-Benzopyran-4-One, 2-(3,4-Dimethoxyphenyl)-3,5-Dihydroxy-7-Methoxy-	888	$C_{24}H_{72}O_{12}Si_{12}$	23.21	Antioxidant, anti-hyperlipidemia (Quranayati *et al.*, 2022)
17	4a,7,7,10a-Tetramethyldodecahydrobenzo[F]Chromen-3-Ol	344	$C_{18}H_{16}O_7$	23.32	Antimicrobial anti-inflamatory (Quranayati *et al.*, 2022)
18	Methyl 3-Methylphenylcarbamate	266	$C_{17}H_{30}O_2$	23.42	Antioxidant (Saravanan *et al.*, 2022)
19	Isochiapin B	165	$C_9H_{11}NO_2$	23.52	Anti-hyperlipidemia (López-Cabeza *et al.*, 2022)
20	Isophthalic Acid, 3,3-Dimethylbut-2-Yl Tridecyl Ester	346	$C_{19}H_{22}O_6$	23.06	Antioxidant, anti-hyperlipidemia (Dawwam *et al.*, 2022)
21	Adipic Acid, Hexadecyl 3-Methylbut-3-Enyl Ester	432	$C_{27}H_{44}O_4$	23.74	Antibacterial (Ahmed *et al.*, 2022)

22	Tetraneurin - A - Diol	438	$C_{27}H50O4$	23.80	Antioxidant, anti-inflammatory (Singh *et al*., 2022)
23	Stigmasta-3,5-Diene	280	$C_{15}H_{20}O_5$	23.84	Antioxidant, anti-inflammatory (Abdel-Motaal *et al*., 2022)
24	Stigmast-5-En-3-Ol, (3.Beta.,24s)-	678	$C_{47}H_82O_2$	23.04	Antioxidant (Madhavan *et al*., 2019)
25	Tetracosamethyl-Cyclododecasiloxane	414	$C_{29}H_{50}O$	24.54	Chemopreventive lipoxygenase inhibitor (Molla *et al*., 2022)

Table 7. Body weight of normal and STZ induced diabetic rats.

Albino Wistar Rats Groups	Change in Body weight (gm)		
	0 day	21th days	45th days
1st	165±1.56	84.67±2.47	194.17±2.99
IInd	180.64±2.17**	161.85±1.77**	124.34±1.93**
IIIrd	173.35±2.12#	167.33±2.45**	187.17±1.98**
IVth	192±2.79#	181.66±2.23**	182.51±1.47**
Vth	173.17±1.65	183.50±2.17**	176.67±1.63**

Footnote: Body weight in normal & STZ induced diabetic rats in non – diabetic rats. Ethanol extract was administered orally in a change of body weight. Significations differences between values as administered by a ANOVA + Tukeys multiple test. Body weight ix expressed in g or as increase in body weight respected today 0 (%) A. No different were found ($P > 0.05$).

Table 8. Plasma glucose values in normal and experimental rats

Albino Wistar Rats Groups	Plasma glucose values in normal & experimental rats		
	0 day	21st day	45th day
1st	95.17±2.15	94.34±1.75	95.5±2.17
II nd	276.34±8.87**	335±12.05**	377.85±11.87**
III rd	262.67±8.55#	194±7.15**	120.7±2.97**
IV th	267.73±0.75	101.5±1.49**	95.17±0.11**
V th	263.51±7.05#	96.5±11.65**	91.5±2.41**

Footnote: Effect of plasma glucose values in normal and experimental rats ethanol extract plasma glucose (A,B) and on the body weight (C) of Albino wistar rats with STZ –Induced diabetic administered. Result represent the mean ± SD of each group (n=6). **< 0.0001 signification different between STZ group and control each group.

Table 9. SGOT, SGPT and ALP levels in normal and experimental rats

Albino Wistar	SGOT (IU/L)		SGPT (IU/L)		ALP (IU/L)	
Rats Groups	0 day	45 th day	0 day	45 th day	0 day	45 th day
1st	62.02±3.47	61.58±1.47	74.35±0.64	75±1.35	74±1.25	76.33±0.81
II nd	152.47±2.63**	222±3.55*	246.89±2.07**	144.57±6.69**	139.69±1.57**	205±1.16**
III rd	140.38±1.69#	101±1.29**	94.86±1.09**	142.34±1.89#	143.32±1.79#	91.4±1.52
IV th	131.25±0.67	157.35±0.62**	124.89±54.7**	127.87±0.09	120.84±0.69	110.3±0.97**
V th	108.39±3.73#	85±1.92#	106±2.19**	73.67±2.37#	124.59±2.37#	80.17±0.85**

Footnote: Effect of normal and experimental animal group of SGOT, SGPT and ALP levels in normal & experimental rats. STZ-Induced and SGOT, SGPT and ALP. Result represent the mean ± SD of each group (n=6). **< 0.0001 signification different among STZ group and control each group.

It was observed that the body weight reduction in the diabetic rat is due to glucose loss, muscle, and fat and protein degradation. Recuperation in total protein content and bw on plant rhizome extract administration at dose range 100-300 mg/kg in the three groups of diabetic rats was observed and

similar results were reported on administration of 300 mg/kg of the extract in rats (Madhavan et al., 2020). In another work rejuvenation in various parts such as sinusoidal spaces, nucleus etc. was observed (Gyawali et al., 2020).

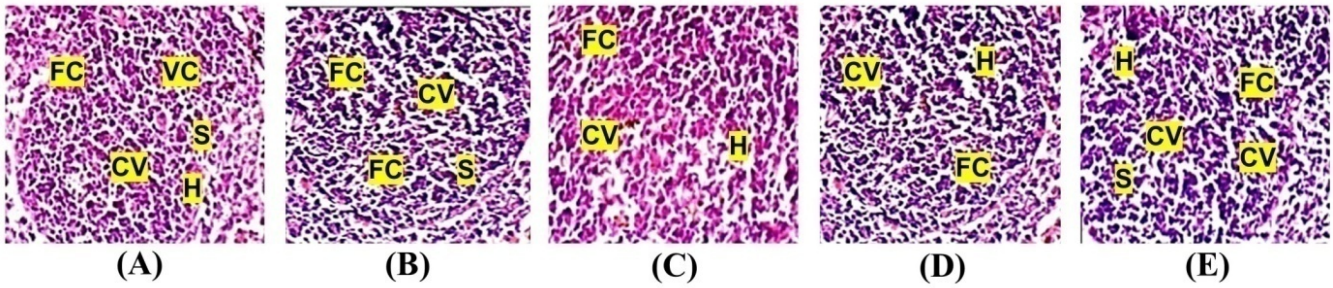

Figure 3. Anti-diabetic Histopathology of wister albino rats. (a) Pancreas Normal rats, (b) Pancreas Diabetic rat's b-cells and damaged cells with an abnormal structure of islet of Langerhans, (c) Diabetic + Glibenclamide (5 mg/kg) shows restoration, (d) Pancreas Diabetic +rhizome plant extract rats at a dose 300 mg/kg, (e) Pancreas Diabetic +rhizome plant extract rats at a dose 500 mg/kg Pancreas showing no pathological alteration of the b-cells of islets of Langerhans and normal appearance of acinar cells. Fc-Fatty Changes, Cv-Central Vein, S-Sinusoids and H-Hepatocyte, pancreas tissue. Cv-Central Vein; Vc-Vacuolation; Fc-Fatty Changes; H-Hepatocyte; S- Sinusoids, H and E magnification X100.

In the present study the presence of bioactive compounds might be correlated to the improvement in histoarchitecture and recovery in diabetic animals (Asmat *et al.*, 2020). It was reported that in particular polyphenols do have the potential for reducing the oxidative stress due to several mechanisms such as inflammation reduction, fatty acids oxidation etc. (Vinotha et al., 2019). Polyphenols also prevents the hepatic fibrosis (Kalakotla *et al.*, 2017), and at a dose range from 100-300 mg/kg do not have a lethal activity on the animals (Soni *et al.*, 2019). Diabetic rats showed the presence of kidney nephropathy (Afolabi *et al.*, 2019) and diabetic animals had an increment in the levels of various markers such as uric acid, urea, and creatinine (Yao *et al.*, 2021). The drug dose at 300 mg/kg can be compared to that of antidiabetic drug glibenclimide.

Significant biochemical alterations were observed on administration of the herbal plant extract on rats on everyday dose; nonetheless, 300 mg/kg dose confirmed extensively greater recuperation in all the biochemical variables that have been determined almost equal to the antidiabetic drug glibenclamide (Gómez *et al.*, 2021). The ordinary animals showed normal histopathological features (Moreno *et al.*, 2020), although the diabetic animal had altered observations such as swelled glomeruli, endothelial lining destruction etc. The distinction between the underlying and last fasting levels of various gathering sun covered a huge height in blood glucose in the diabetic benchmark group contrasted with ordinary (Fatunde *et al.*, 2020).

The histological assessment of the liver examples from control creatures uncovered typical hepatic engineering and polyhedral hepatocytes, notwithstanding, the liver of creatures regulated with paracetamol alone showed scattered hepatocytes, multicentral zone corruption, clogged veins and greasy debasement (Ghosh *et al.*, 2014). Albeit the liver tissues of creatures treated with silymarin and paracetamol showed gentle changes in liver engineering and clogged vein, no indications of harm were seen in liver tissue got from the creatures treated with *T. foliolosum* extricate and paracetamol (Keerthana *et al.*, 2013). Silmarin and its explo-ration bunch detailed that alkaloids, primarily having a place with the class of isoquino-line alkaloids are the significant auxiliary metabolites found in *Thalictrum* species; a large part helpful capability of the plant would thus be able to be ascribed (El-

Beshbishy *et al.*, 2016). However, further studies have to be performed for future exploration. Insulin deficiency has been estimated to be the most probable reason for the elevated SGPT, SGOT, ALP and cholesterol level in STZ induced diabetic rats. The formulation did not show any other toxic effects, probably due to the decreased quantity of each drug in the formulation, also associated with its ability to restore histological and cell survival.

Conclusion

The current study aimed to evaluate the anti-hyperglycaemic properties of ethanolic extract from *Costus spicatus* (jacq) on Streptozotocin-induced diabetic male albino Wistar rats. A total of 25 compounds were identified by GC-MS, all reported to possess several biological activities. Histophathological analysis confirmed that pancreas diabetic rhizome plant extract albino Wister rats at a dose of 500 mg/kg showed no pathological alteration of the β-cells of islets of Langerhans and normal appearance of acinar cells. In arrangement of hepatic cords, nucleus development and sinusoidal spaces appear normal on histopathological examination. Serum and cellular proteins had a significant reduction. Body weight reduction in the diabetic rat is due to glucose loss, muscle, and fat and protein degradation. Decreased glucose level, serum glutamic pyruvic transaminase (SGPT), Serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP), in the experiments confirmed that selected plant a potential tool for antidiabetic treatments.

Acknowledgement: This work supported by Department of Community Medicine, Saveetha Institute of Medical and Technical Sciences (SIMATS) Chennai - Tamil Nadu, India.

Disclosure statement: *Conflict of Interest:* The authors declare that there are no conflicts of interest.
Compliance with Ethical Standards: Animal experiments were performed by following the guideless provided by CPCSEA and IAEC (Reg. No: 685/PO/Re/S/2002; dated 21 August 2002 and KMCRET/Ph.D/22/2018-19)

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