

IN VITRO ANTIOXIDANT ACTIVITY AND HYPOGLYCEMIC EFFICACY OF THE LEAF, STEM, AND RHIZOME EXTRACTS OF *Costus igneus* Nak (COSTACEAE) IN ALLOXAN INDUCED DIABETIC RATS



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ABSTRACT

Costus igneus has a folk reputation in West Africa for the treatment of diabetes. Nevertheless, from our investigations, the scientific validation of this folkloric claim has not been properly documented. The study was designed to evaluate the antidiabetic potentials and *in vitro* antioxidant capacity of the leaves, stems and rhizomes of *C. igneus* using ethanol as the extraction solvent. Phytochemical screening of the extracts was performed using standard analytical procedures. Saponins, tannins, flavonoids, alkaloids, and cardiac glycosides were detected in the leaves, stems, and rhizomes of *C. igneus*. The median lethal dose (LD₅₀) of the leaf, stem, and rhizome extracts in mice were 2958.04, 1936.49, and 5000 mg/kg, respectively. The treatment of alloxan-induced diabetic rats with the extracts of the leaves, stems, and rhizomes caused a significant reduction ($P < 0.05$) in fasting blood glucose levels (44.13, 56.21, and 61.12%), respectively in acute study, and 76.85, 69.95, and 79.63% reduction in prolong treatment (2 weeks). The leaf and rhizome extract also demonstrated good antioxidant activity on 2, 2-diphenyl-1-picrylhydrazyl radicals (61 and 62% inhibition, 100 µg/mL) and ferric reducing capacity (absorbance, 0.541 and 0.459, 100 µg/mL), respectively. The leaves, stems, and rhizomes of *C. igneus* exhibited good hypoglycaemic activity which supports their folkloric claims in the management of diabetes mellitus. The rhizomes extract showed the least toxicity and the most effective in the reduction of fasting blood glucose levels. This is the first comparative evaluation of the leaves, stems, and rhizomes of *C. igneus* in a study. The findings suggest the use of the rhizomes as a better substitute than the leaves popularly employed in herbal preparations or a combination of all the plant parts for an effective herbal treatment. The antioxidant capacity of this plant extracts may also be involved in the inhibition of oxidative processes implicated in diabetic complications.

KEYWORDS: Costaceae, *Costus igneus*, Lethal dose, Antidiabetic agent, Antioxidant.

INTRODUCTION

Diabetes mellitus is a complex and a multifarious group of disorders that disturbs the metabolism of carbohydrates, fat and protein. It is caused by insufficient or inefficient insulin secretory response and it is characterized by increased blood glucose levels known as hyperglycemia (Ezurike and Prieto, 2014). Type 2 diabetes mellitus, or non- insulin dependent diabetes mellitus is the most common type and its initial stage is treated with oral antidiabetics. Though antihyperglycemic agents with different mechanisms of action are commercially available, their adverse effects such as hepato-renal toxicity, diabetic retinopathy, diabetic nephropathy and diabetic neuropathy are pronounced (Hedge et al., 2014). Thus, the discovery of new and safer medicines is deserving.

Costus igneus Nak (syn. *Costus pictus* D. Don), commonly known as spiral flag, is a member of the Costaceae. It is a perennial, upright, spreading plant reaching about two feet tall, with spirally arranged leaves and attractive flowers (Hedge et al., 2014). Folkloric claims indicate that *C. igneus* is capable of having potent cure for diabetes, hence diabetics consume one leaf daily to keep their blood glucose levels low (Devi and Urooj, 2008; Elavarasi and Saravanan, 2012). The leaf of this herbal plant (popularly known as “Insulin Plant”) is believed to help in the buildup of insulin by strengthening beta cells of pancreas in the human body (Hedge et al., 2014). A number of researches have been carried out to evaluate the anti-diabetic potentials of the leaves (Bhat et al., 2010; Devi and Urooj, 2008; Mani et al.,

2010; Shetty et al., 2010a; Suganya et al., 2012), rhizomes (Kalailingam et al., 2011), and an insulin-like protein (Joshi et al., 2013; Hardikar et al., 2016) of *C. igneus* using animal models. A clinical study on diabetic patients revealed that, after 15 days of consuming the leaves of *C. igneus*, a sharp decrease in fasting blood sugar level was observed (Shetty et al., 2010b). The antioxidant activities of the leaves of *C. igneus* have also been reported (Jayasri et al., 2009; Majumdar and Parihar, 2012). α -Tocopherol and ergastanol are chemical constituents identified in the leaves (George et al., 2007), lupeol and stigmaterol in the stems (Manjula et al., 2012), quercetin and diosgenin in the rhizomes (Kalailingam et al., 2011) of the plant.

In Nigeria -cum- West Africa, *Costus* species including *C. igneus* are also employed in ethnomedicine for the management of diabetes mellitus (Ajibesin et al., 2008; Elavarasi and Saravanan, 2012; Jeroh et al., 2020) however, reports are only available for the Indian grown *C. igneus*. To the best of our knowledge, there is paucity of data on the antidiabetic and antioxidant potentials of the stem and rhizome of *C. igneus*. It is a known fact that climatic and environmental change factors affect plant secondary metabolites and plant insect interactions (Jamieson et al., 2017). Furthermore, variation in agronomic conditions, season, climatic factor, water availability, light, and CO₂ are known to significantly affect content and profile of phytochemicals (Björkman et al., 2011). Based on the aforementioned and as an integral part of our scientific validation of indigenous plants with ethno-medicinal claims

(Thomas *et al.*, 2021), this study is carried out in order to validate the folkloric claims attributed to *C. igneus* in the management of diabetes mellitus by evaluating the antidiabetic activities of the ethanol extracts of the leaves, stems, and rhizomes, in addition to their antioxidant potentials.

MATERIALS AND METHODS

Plant Collection, Authentication and Preparation of Extracts

The leaves, stems and rhizomes of *C. igneus* were collected from the ravine, near the University of Uyo town campus in April 2020. The plant was identified by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, where a voucher specimen (UUH 4061) was deposited. The fresh samples were washed and drained, then pulverized and macerated in ethanol (99%) in a glass extraction jar for 72 hours, and re-macerated to obtain maximum yield. The filtrates were concentrated using a rotary evaporator at 40 °C to obtain the different plant extracts.

Animals

Adult Swiss albino mice (15–22 g) of both sexes and adult Wistar rats of both sexes (120 – 170 g) were procured from the University of Uyo animal house. The animals were housed in standard cages and fed with standard livestock pellets (Guinea Feed) and water *ad libitum*, and maintained under standard laboratory conditions (23–25 °C; 12h/12h light/dark cycle). The animals were used for the experiment after 24 h fast and were only deprived of water during the experiment. Animal handling and experimental procedures were performed strictly in accordance with international ethical guidelines concerning the care and use of laboratory animals (Kilkenny *et al.* 2010; NRC Publication, 2011). All the experiments were carried out under the approval of the ethical committee of the Faculty of Pharmacy, University of Uyo (ethics approval no. UU/FPEC/ 2020/018).

Determination of Median Lethal Dose (LD₅₀)

The method of Lorke (1983) was used to determine the LD₅₀. Adult Swiss albino mice, randomized into three groups of three mice each for the phase 1, and three groups of one mouse each for phase 2, were administered the ethanol extracts of leaves, stems and rhizomes, orally. This involved the administration of different doses of 100, 1000 and 1500 mg/kg of the leaf, stem and rhizome extracts in the first phase; 2500, 3500 and 5000 mg/kg in the second phase, respectively. The mice were observed post-treatment for physical signs of toxicity such as restlessness, dullness, agitation, increase respiration, writhing, and death. The number of deaths per group within 24 h was recorded. The median lethal dose (LD₅₀) was calculated as the square root of the product of the maximum dose that kill no animal and the minimum dose that kill all the animals in the group (Ozolua and Bafor, 2019).

Inducement of Diabetes in Rats

The male rats were fasted for 24 h and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of alloxan monohydrate (150 mg/kg) in ice cold 0.9% saline (NaCl) solution. The animals were given 2 mL of 5% dextrose solution using orogastric tube immediately

after induction to overcome the drug induced hypoglycemia. Seventy-two hours later, rats with blood glucose levels (BGLs) above 200 mg/dL were considered diabetic and selected for the antidiabetic experiment (Bhat *et al.*, 2010).

Evaluation of Anti-diabetic Activity

The animals were divided into eleven groups of five rats each and treated as follows:

Group 1: Diabetic rats treated with 10 mL/kg body weight of distilled water orally for 14 days.

Group 2: Diabetic rats treated with 10 mg/kg body weight of glibenclamide orally for 14 days.

Group 3: Diabetic rats administered with 300 mg/kg body weight of leaves extract orally for 14 days.

Group 4: Diabetic rats administered with 600 mg/kg body weight of leaves extract orally for 14 days.

Group 5: Diabetic rats administered with 890 mg/kg body weight of leaves extract orally for 14 days.

Group 6: Diabetic rats administered with 190 mg/kg body weight of stem extract orally for 14 days.

Group 7: Diabetic rats administered with 390 mg/kg body weight of stem extract orally for 14 days.

Group 8: Diabetic rats administered with 580 mg/kg body weight of stem extract orally for 14 days.

Group 9: Diabetic rats administered with 500 mg/kg body weight of rhizome extract orally for 14 days.

Group 10: Diabetic rats administered with 1000 mg/kg body weight of rhizome extract orally for 14 days.

Group 11: Diabetic rats administered with 1,500 mg/kg body weight of rhizome extract orally for 14 days.

The fasting blood glucose levels (BGLs) of all the rats were recorded at regular intervals during the experimental period. The BGLs for the acute study were monitored after 1, 2, 3, 5 and 7 h of administration of three doses of each extract and at the end of 1, 7 and 14 days for prolonged treatments. The BGLs were monitored in the blood of the diabetic rats by tail tipping method. The blood was dropped on the dextrostix reagent pad. This was inserted into microprocessor digital blood glucometer and the readings were recorded.

Phytochemical Evaluation

Standard methods for phytochemical screening of alkaloids, flavonoids, saponins, tannins, cardiac glycosides and anthraquinones were used to evaluate the leaf, stem and rhizome extracts of *C. igneus* (Harbone, 1984; Trease and Evans, 2009).

Determination of DPPH Radical Scavenging Activity

The plant extracts and ascorbic acid were prepared in methanol (20–100 µg/mL) and evaluated according to the method adopted by Essien and Thomas (2018). DPPH (0.1 mM, 1 mL) in methanol was added to different concentrations of the samples and ascorbic acid (3 mL each) in test tubes. The mixture was vortexed and incubated in a dark chamber for 30 min, and the absorbance (As) measured at 517 nm. The assay was carried out in triplicate and the results expressed as mean values ± standard error of mean. Percentage inhibition = $[(A_0 - A_s) / A_0] \times 100$

Where A_0 is the absorbance of control reaction and A_s is the absorbance of the test sample or standard sample (ascorbic acid).

Determination of Ferric Reducing Antioxidant Power

The reducing power of the extracts was determined according to the method of Yen and Chen (1995). Various concentrations of the plant extracts and ascorbic acid (2.5 mL) were mixed with sodium phosphate buffer (pH 6.6, 200 mM, 2 mL) and 30 mM potassium ferricyanide (2 mL). The mixture was incubated at 50 °C for 20 min, followed by the addition of trichloroacetic acid (10% w/w, 2 mL); then centrifuged at 3000 rpm for 10 min. The upper layer of the resultant solution (5 mL) was mixed with deionised water (5 mL) and ferric chloride (0.1% w/v, 1 mL). The absorbance was measured at 700 nm. The assays were carried out in triplicate and the results were expressed as mean ± SEM.

Statistical Analysis

Data were analyzed by one-way and two-way analysis of variance (ANOVA) followed by “turkey’s multiple comparison” and “Bonferroni” posttests (‘GraphPad Prism’ software Inc. version 5.03, La Jolla, CA, USA), respectively. Values were expressed as mean ± SEM and significance relative to control were considered at $p < 0.05$, $p < 0.01$ and $p < 0.001$.

RESULTS AND DISCUSSION

The median lethal dose (LD₅₀) of the extracts of *C. igneus* in mice is shown in Table 1.

Table 1: Median lethal dose (LD₅₀) of extracts of *C. igneus* in mice.

Samples	Phase	No of Mice	Dose (mg/kg)	Mortality	LD ₅₀ (mg/kg)
Leaves extract	1	3	100	0/3	2958.04
		3	1000	0/3	
		3	1500	0/3	
	2	1	2500	0/1	
		1	3500	1/1	
		1	5000	1/1	
Stem extract	1	3	100	0/3	1936.49
		3	1000	0/3	
		3	1500	0/3	
	2	1	2500	1/1	
		1	3500	1/1	
		1	5000	1/1	
Rhizome extract	1	3	100	0/3	5000
		3	1000	0/3	
		3	1500	0/3	
	2	1	2500	0/1	
		1	3500	0/1	
		1	5000	0/1	

The LD₅₀ for oral administration were 2958.04 mg/kg, 1936.46 mg/kg, and 5000 mg/kg for leaves, stems, and rhizomes extracts, respectively. A comparison of the toxicity index of the extracts revealed that the rhizome extract of *C. igneus* would be the safest for human intake on the basis of their LD₅₀ values, via oral route, using animal models. The LD₅₀ for oral administration of the leaves extract of *C. igneus* in this study is 2958.04 mg/kg; relatively higher than that obtained for the aqueous leaves extract of *C. pictus* (1000

mg/kg) as reported by Remya and Daniel (2012), which showed no signs of toxicity throughout the study. Conversely, Bhat *et al.* (2010) indicated that the ethanol leaf extract of *C. igneus* (50 mg/kg to 5000 mg/kg) did not show signs of significant toxicity for first 4 hours, followed by a daily observation for 14 days and still no mortality was observed. However, as far as we know, this is the first report on the toxicity profiles of the stem and rhizome of *C. igneus*.

The hypoglycaemic effects of the plant extracts on blood glucose levels of alloxan induced diabetic rats during acute and prolong study are shown in Tables 2 and 3. The dosage of extracts employed in this study was calculated as 1/10th, 2/10th and 3/10th of the LD₅₀, to represent low, median, and high doses, respectively. The leaves extract produced significant ($p < 0.05$) dose dependent reduction in the fasting blood glucose levels (FBGL) in the diabetic rats during acute study when compared to negative control. The percentage reduction in FBGL (300, 600, and 890 mg/kg) were 44.53, 44.63 and 44.13%, respectively, compared to glibenclamide (69.23%) (Table 2). The highest activity of the leaves extract was observed on prolong treatment (14 days) which produced a remarkable reduction in FBGL in diabetic rats. The percentage reductions were 63.34%, 73.42%, and 76.85% at dosage levels of 300, 600, and 890 mg/kg, respectively, compared to glibenclamide (82.31%); these were significant ($P < 0.001$) when compared to negative control (Table 3). Similarly, a number of researches have consistently demonstrated the hypoglycaemic efficacy of the leaves extract of *C. igneus* in diabetic rats (200-500 mg/kg) (Devi and Urooj, 2008; Bhat *et al.*, 2010; Mani *et al.*, 2010; Shetty *et al.*, 2010a; Suganya *et al.*, 2012). Nevertheless, a relative high dose (2 g/kg) of the aqueous leaf extract of *C. igneus* has also been reported (Jayasri *et al.*, 2008).

The stem extract also demonstrated significant reduction in FBGL in diabetic rats. In the acute study (7 h), the percentage reductions in FBGL were 27.17, 39.69, and 56.21%, at the doses of 190, 390, and 580 mg/kg, respectively. These reductions were significant at $P < 0.01$ when compared with negative control; however, it was not comparable to glibenclamide (69.23%) (Table 2). On prolong study (14 days), there was significant ($p < 0.01$) reduction in FBGL (17.59, 59.07, and 69.95%) at 190, 390 and 580 mg/kg when compared to control; again, it was not comparable to glibenclamide (82.31%) (Table 3).

The rhizome extract exhibited the highest reduction efficacy in FBGL when compared with leaves and stem extracts of *C. igneus*. The relative high doses of the rhizomes extract in this study are consistent with the LD₅₀ results (>5000 mg/kg) (Table 1). In the acute study (7 h), there was significant ($p < 0.01$) percentage reduction in FBGL of 41.96, 53.35, and 61.19% at the dosage level of 500, 1000, and 1500 mg/kg, respectively, when compared to negative control. This reduction was not comparable to that of glibenclamide (69.23%) as shown in Table 2. On prolong treatment, there was significant ($p < 0.001$) reduction in FBGL of 76.11, 76.25, and 79.63% at the dosage level of 500, 1000 and 1500 mg/g, and this was comparable to that of glibenclamide (82.31%) (Table 3). Kalailingam *et al.* (2011) also showed

that the rhizome extract of *C. igneus* significantly reduced the blood glucose levels in diabetic rats.

Table 2: Antidiabetic effect of extracts of *C. igneus* on blood glucose level of alloxan induced diabetic rats during acute study.

Treatment	Dose (mg/kg)	Blood Glucose Level mg/dL					
		0 Hr	1 Hr	2 Hr	3 Hr	5 Hr	7 Hr
Control	10	405.6±16.04	401.6±14.82	395.0±14.36	375.2±18.28	371.4±20.27	405.6±7.35
Leaf Extract	10	343.0±35.90	236.2±37.62 ^a	217.6±31.61 ^a	194.2±33.39 ^a	156.2±27.92 ^b	124.8±18.68 ^c
	300	333.60±47.30	254.8±40.88 ^{ns}	231.8±35.00 ^a	248.4±38.26 ^{ns}	238.0±37.62 ^{ns}	225.0±33.23 ^a
	600	358.0±47.77	272.8±24.93 ^{ns}	249.6±23.07 ^{ns}	251.4±24.84 ^{ns}	236.8±24.31 ^{ns}	224.6±25.66 ^a
	890	407.2±39.96	320.0±28.73 ^{ns}	309.0±24.73 ^{ns}	298.6±24.70 ^{ns}	248.6±24.70 ^{ns}	226.6±9.85 ^{ns}
Stem Extract	190	339.2±83.48	272.8±58.74 ^{ns}	277.8±57.52 ^{ns}	284.4±57.84 ^{ns}	287.4±58.14 ^{ns}	295.4±60.60 ^{ns}
	390	338.8±56.94	286.0±46.59 ^{ns}	275.0±42.68 ^{ns}	266.8±40.13 ^{ns}	259.0±38.63 ^{ns}	244.6±36.12 ^{ns}
	580	245.5±64.80	229.8±28.94 ^a	220.0±27.77 ^a	211.0±26.64 ^a	195.4±28.48 ^a	177.6±27.45 ^b
Rhiz. Extract	500	328.6±63.49	311.0±64.11 ^{ns}	262.8±62.17 ^{ns}	254.6±60.64 ^{ns}	238.0±62.48 ^{ns}	235.4±61.84 ^{ns}
	1000	289.0±91.50	237.6±78.65 ^a	204.6±62.78 ^b	203.6±59.53 ^a	195.6±57.18 ^a	189.2±56.02 ^b
	1500	290.0±90.63	222.2±71.33 ^a	209.6±71.52 ^b	182.0±57.63 ^b	147.6±50.22 ^c	157.4±50.28 ^c

Data are expressed as Mean ± SEM, significant at ^aP < 0.05, ^bP < 0.01 and ^cP < 0.001; and ns = not significant, when compared to control, (n=5), Rhiz. = Rhizome.

Table 3: Antidiabetic effect of *C. igneus* extracts on blood glucose levels of alloxan induced diabetic rats during prolonged study.

Treatment	Dose (mg/kg)	Blood Glucose Level mg/dL			
		Day 0	Day 1	Day 7	Day 14
Control	10 mL/kg	405.6±16.03	405.6±17.35	448.0±48.59	402.6±73.97
Leaf Extract	10	343.0±35.90	113.4±15.67 ^c	85.20±5.78 ^c	71.2±6.43 ^c
	300	333.6±47.30	207.0±36.11 ^b	172.6±26.84 ^c	147.6±25.17 ^c
	600	358.0±47.77	218.0±28.90 ^b	144.6±25.70 ^c	107.0±9.92 ^c
Stem Extract	890	407.2±39.96	172.0±16.40 ^c	121.6±5.08 ^c	93.2±4.92 ^c
	190	339.2±83.48	330.0±68.17 ^{ns}	337.0±62.87 ^{ns}	331.8±62.39 ^{ns}
	390	338.8±56.94	234.4±36.50 ^a	206.4±33.29 ^c	164.8±35.73 ^c
Rhizome Extract	580	245.8±64.80	164.40±24.01 ^c	138.2±10.56 ^c	121.0±5.54 ^c
	500	328.6±63.49	229.0±63.42 ^a	177.2±52.86 ^c	96.2±3.94 ^c
	1000	289.0±91.50	133.2±16.35 ^c	98.4±2.99 ^c	95.6±3.01 ^c
	1500	290.0±90.63	128.2±25.13 ^c	89.0±6.03 ^c	82.0±4.32 ^c

Data are expressed as Mean ± SEM, significant at ^aP < 0.05, ^bP < 0.01 and ^cP < 0.001; and ns = not significant, when compared to control; (n=5).

The preliminary phytochemical screening result of the plant extracts is shown in Table 4. The presence of saponins, tannins, flavonoids, alkaloids, and cardiac glycosides are indicated in the three extracts. Anthraquinones were not detected in all the plant parts. Reports have shown the identification of steroids and flavonoids in the leaves of *C. igneus*, in addition to alkaloids, tannins, and saponins (George et al., 2007; Shankarappa et al., 2011; Peasari et al., 2018). However, a number of hyphenated techniques such as LC-MS and HPTLC have been employed in the identification of lupeol and stigmaterol in the leaves (Manjula et al., 2016) and quercetin and diosgenin (Kalailingam et al., 2011) in the rhizomes of *C. igneus*.

Table 4: Phytochemical screening of *C. igneus*.

Phytochemical	Leaf	Stem	Rhizome
Saponin	+	+	+
Tannin	+	+	+
Flavonoid	+	+	+
Alkaloid	+	+	+
Cardiac Glycosides	+	+	+
Anthraquinone	-	-	-

The DPPH radical scavenging activity and ferric ion reducing power of the plant extracts are shown in Figures 1 and 2 respectively. The plot in Figure 1 indicates the scavenging ability as percent inhibition at various concentrations and that the scavenging effect was concentration dependent. The extracts of the leaves, stems and rhizomes demonstrated the scavenging effect by acting as hydrogen atoms or electron donors in the conversion of the stable purple coloured DPPH to the reduced yellow coloured DPPH. The leaf and rhizome extracts showed good and significant (p<0.001) radical scavenging effect (61 and 62 % inhibition, respectively; 100 µg/mL) whereas the antioxidant potential of the stem extract was relatively lower (52% inhibition). The antioxidant efficacy of these extracts was not comparable to that of ascorbic acid (87%; 100 µg/mL) used as the standard.

The ferric ion reducing power (FRAP) of the antioxidants in the leaves, stems, and rhizomes extracts (Figure 2) against the oxidative consequences of reactive oxygen species, *in vitro*, is depicted as the resultant absorbance (700 nm) at various concentrations. The reducing potential of ascorbic acid (absorbance 1.288; 100 µg/mL) doubled the ferric ion reducing power of the leaves (0.451), rhizomes (0.459) and stem (0.431) extracts. The antioxidant activity of the extracts implicates the plant constituents' ability to reduce the (Fe³⁺)

to (Fe²⁺) by electron transfer. Similarly, the leaves and rhizomes of *C. pictus* have been shown to exhibit good antioxidant activity (89.5% and 90.0% inhibition; 400 µg/mL) using DPPH and FRAP models (Jayasri *et al.*, 2009); the higher percent inhibition of the extracts in their study may be as a result of the 400 µg/mL extract concentration compared with a 100 µg/mL used in our antioxidant assay. The methanol leaf extract of *C. pictus* has

also been reported to cause significant increase in superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, vitamin A, vitamin C, vitamin E and reduced glutathione (Sethumathi *et al.*, 2009); likewise, the stem extract has been shown to exhibit antioxidant activity against oxidative protein damage, *in vitro* (Majumdar and Parihar, 2012).

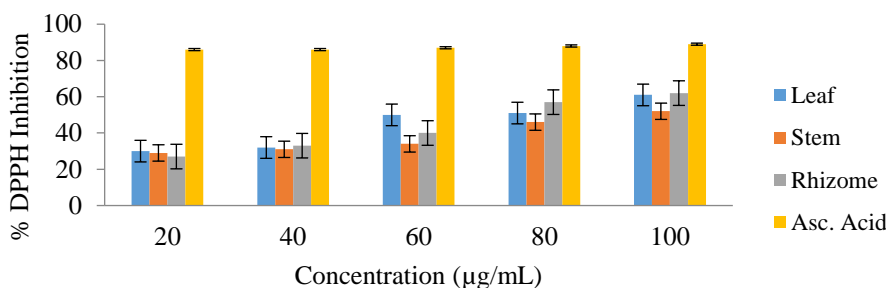


Fig. 1. DPPH assay of the leaf, stem and rhizome extracts of *Costus igneus*

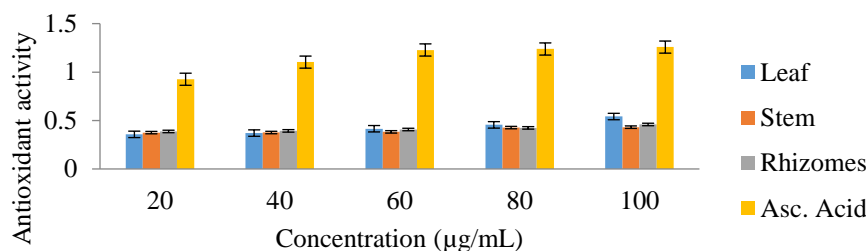


Fig. 2. Ferric ion reducing power assay of the leaf, stem and rhizome extracts of *C. igneus*

CONCLUSIONS

The ethanol extract of rhizomes of *C. igneus* showed no acute toxicity index, whereas the leaf and stem extracts induced different levels of toxicity with increase doses in mice. The rhizome extracts also exhibited good hypoglycaemic effects in alloxan induced diabetic rats compared with the leaf and stem extracts. The leaf, stem, and rhizome extracts demonstrated good antioxidant activities; however, the least DPPH radical scavenging effect and ferric ion reduction capacity were observed in the stem extract. The findings suggest that the rhizomes of *C. igneus* is a better substitute for the frequently used leaves therapy or a combination of all the parts of *C. igneus* in herbal formulations may produce better outcomes in the treatment of diabetes mellitus. Furthermore, the antioxidants in the extracts of *C. igneus* may also be involved in the inhibition of oxidative processes implicated in diabetic complications.

REFERENCES

Ajibesin, K. K., Ekpo, B. A., Bala, D. N., Essien, E. E. and Adesanya, S. A. (2008). Ethnobotanical survey of Akwa Ibom State of Nigeria. *Journal of Ethnopharmacology*, 115: 387–408.

Bhat, V., Kamat, A., Abuti, N. and Patil, M. B. (2010). Antidiabetic activity of insulin plant (*Costus igneus*) leaf extract in diabetic rats. *Journal of Pharmaceutical Research*, 3(3): 603–611.

Björkmana, M., Klingena, I., Birch, A. N. E., Bones, A. M., Bruce, T. J. A., Johansene, T. J., Meadow, R., Møllmann, J., Smart, R. E. and Stewart, D. (2011). Phytochemicals of Brassicaceae in plant protection and human health – influences of climate, environment and agronomic practice. *Phytochemistry*, 72(7): 538–556.

Devi, V. D. and Urooj, A. (2008). Hypoglycemic potential of *Morus indica* L. and *Costus igneus*: A preliminary study. *Indian Journal of Experimental Biology*, 46: 614–616.

Elavarasi, S. and Saravanan, K. (2012). Ethnobotanical study of plants used to treat diabetes by tribal people of Kolli Hills, Namakkai District, Tamilnadu, Southern India. *International Journal of Pharmaceutical Technology Research*, 4: 404–411.

Essien, E. E. and Thomas, P. S. (2018). Toxicity, hypoglycemic and antioxidant potentials of *Massularia accuminata* stem. *Journal of Pharmacognosy and Phytochemistry*, 7(5): 1222–1226.

Ezuruike, U. F. and Prieto, J. M. (2014). The use of plants in the traditional management of diabetes in Nigeria: Pharmacological and toxicological considerations. *Journal of Ethnopharmacology*, 155(2): 857–924.

George, A., Thankamma, A., Rema Devi, V. K. and Fernandez, A. (2007). Phytochemical investigation of insulin plant (*Costus pictus*). *Asian Journal of Chemistry*, 19: 3427–30.

- Harborne, J. E. (1984). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis* (3rd Edition). Netherlands: Springer.
- Hardikar, M. R., Varma, M. E., Kulkarni, A. A., Kulkarni, P. P. and Joshi, B. N. (2016). Elucidation of hypoglycemic action and toxicity studies of insulin-like protein from *Costus igneus*. *Phytochemistry*, 124: 99-107.
- Hegde, P. K., Rao, H. A. and Rao, P. N. (2014). A review of insulin plant (*Costus igneus* Nak). *Pharmacognosy Reviews*, 8(15): 67-72.
- Jamieson, M. A., Burkle, L. A., Manson, J. S., Runyon, J. B., Trowbridge, A. M. and Zientek, J. (2017). Global change effects on plant–insect interactions: The role of phytochemistry. *Current Opinion in Insect Science*, 23: 70-80.
- Jayasri, M. A., Gunasekaran, S., Radha, A. and Mathew, T. L. (2008). Anti-diabetic effect of *Costus pictus* leaves in normal and streptozotocin-induced diabetic rats. *International Journal of Diabetes in Developing Countries*, 16: 117–22.
- Jayasri, M. A., Mathew, L. and Radha, A. (2009). A report on the antioxidant activity of leaves and rhizomes of *Costus pictus*. *International Journal of Integrative Biology*, 5: 20-26.
- Jeroh, E., Awhin, E. P. and Eyikimiaghan, E. A. (2020). Glutathione reductase and malondialdehyde activity in alloxan-induced diabetic rats treated with *Costus lucanusianus*. *Research Journal of Medicinal Plants*, 14: 144-148.
- Joshi, B. N., Munot, H., Hardikar, M. and Kulkarni, A. A. (2013). Orally active hypoglycemic protein from *Costus igneus*: An *in vitro* and *in vivo* study. *Biochemistry and Physiology: Open Access*, 436(2): 278-282.
- Kalailingam, P., Sekar, A. D., Samuel, J. S., Gandhirajan, P., Govindaraju, Y., Kesavan, M., Kaliaperumal, R., Shanmugan, K. and Tamilmani, E. (2011). The efficacy of *Costus igneus* rhizomes on carbohydrate metabolic, hepatoprotective, and antioxidative enzymes in streptozotocin-induced diabetic rats. *Journal of Health Science*, 57: 37-46.
- Kilkenny, C., Browne, W. J., Cuthill, I. C., Emerson, M. and Altman, D. G. (2010). Improving bioscience reporting: The ARRIVE guidelines for reporting animal research. *PLoS Biology*, 8(6): 100–412.
- Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology*, 54: 275-285.
- Majumdar, M. and Parihar, P. S. (2012). Antibacterial, antioxidant, and antiglycation potential of *Costus pictus* from southern region, India. *Asian Journal of Plant Science and Research*, 2: 95–101.
- Mani, P., Kumar, A. R., Bastin, T. M., Jenifer, S. and Arumugam, M. (2010). Comparative evaluation of extracts of *Costus igneus* (or *Costus pictus*) for hypoglycemic and hypolipidemic activity in alloxan-diabetic rats. *International Journal of Pharmacy and Technology*, 2: 183–195.
- Manjula, K., Pazhanichamy, K., Kumaran, S., Eevera, T., Dale, K. C. and Rajendran, K. (2012). Growth characterization of calcium oxalate monohydrate crystals influenced by *Costus igneus* aqueous stem extract. *International Journal of Pharmaceutical and Pharmaceutical Sciences*, 4(Suppl 1): 261-270.
- National Research Council (US). (2011). *Guide for the Care and Use of Laboratory Animals* (8th ed.). Washington, DC: National Academic Press (US), 246p.
- Ozolua, R. I. and Bafor, E. E. (2019). *A Handbook of Techniques in Experimental Pharmacology*. Nigeria: Mindex Press Ltd., pp. 277–280.
- Peasari, J. R., Motamarri, S. S., Varma, K. S., Anitha, P. and Potti, R. B. (2018). Chromatographic analysis of phytochemicals in *Costus igneus* and computational studies of flavonoids. *Informatics in Medicine Unlocked*, 13: 34–40.
- Remya, R. and Daniel, M. (2012). Phytochemical and pharmacognostic investigation of antidiabetic *Costus pictus* D. Don. *International Journal of Pharmacy and Biomedical Research*, 3: 30-39.
- Sethumathi, P. P., Nandhakumar, J., Sengottuvelu, S., Duraisam, R., Karthikeyan, D. and Ravikumar, V. R. (2009). Antidiabetic and antioxidant activity of methanolic leaf extracts of *Costus pictus* D. Don in alloxan-induced diabetic rats. *Pharmacologyonline*, 1: 1200-1313.
- Shankarappa, L., Gopalakrishna, B., Jagadish, N. R. and Siddalingappa, G. S. (2011). Pharmacognostic and phytochemical analysis of *Costus ignitus*. *Internationale Pharmaceutica Scientia*, 1: 36-41.
- Shetty, A. J., Rejeesh, D. C., Nair, V. and Kuruvilla, M. (2010a). Effect of the insulin plant (*Costus igneus*) leaves on dexamethasone-induced hyperglycemia. *International Journal of Ayurveda Research*, 2: 100-102.
- Shetty, J., Parampalli, S. M., Bhandarkar, R. and Kotian, S. (2010b). Effect of insulin plant (*Costus igneus*) leaves on blood glucose levels in diabetic patients: A cross-sectional study. *Journal of Clinical and Diagnostic Research*, 4: 2617–2621.
- Suganya, S., Narmadha, R., Gonasakrishnan, V. K. and Devaki, K. (2012). Hypoglycemic effect of *Costus igneus* on alloxan-induced type 2 diabetes mellitus in albino rats. *Asian Pacific Journal of Tropical Disease*, 2(2), 117-123.
- Thomas, P., Essien, E., Udoh, A., Archibong, B., Akpan, O., Etukudo, E., Leo, M., Eseyin, O., Flamini, G. and Ajibesin, K. (2021). Isolation and characterization of anti-inflammatory and analgesic compounds from *Uapaca staudtii* Pax (Phyllanthaceae) stem bark. *Journal of Ethnopharmacology*, 269: 113737.
- Trease, G. E. and Evans, W. C. (2009). *Textbook of Pharmacognosy* (16th Edition). New York: Saunders Limited. pp. 231-233.
- Yen, G. C. and Chen, H. Y. (1995). Comparison of antimutagenic effect of various tea extracts (green, oolong, pouching, and black tea). *Journal of Food Protection*, 57: 54–58.