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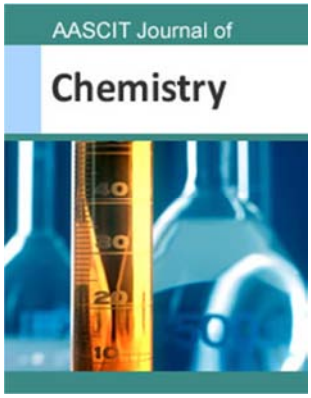

The Physicochemical, Toxicity and Anti-Diabetic Effect of *Costus afer* Ker Gawl. (Costaceae) Leaf Methanol Extract and Snail Slime on Alloxan Induced White Swiss Albino Rat

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Abstract

The wisdom of traditional knowledge of medicinal plants is a great potential for research and discovery of new drugs to fight modern diseases like diabetes. In Nigeria, *Costus afer* is a medicinal plant with many uses which widely covers the treatment and management of many ailments. In this study, some physicochemical, toxicity and anti-diabetic effects of snail slime and *Costus afer* leaf methanol extract on fasting blood glucose level of a Swiss albino rat was investigated. Moreso, the work of "[3]" gave support to the current study of the *Costus afer* leaf methanol extract and snail slime on alloxan induced white Swiss albino rat investigated for 21 days on oral administration of the extract. The solubility status of the snail slime was evaluated using different organic solvents, mineral acids and alkalis. The preliminary phytochemical screening of methanol, acetone, and water extracts of the *Costus afer* leaves was conducted with indication of the presence of alkaloid, phenol, flavonoid, cardenoloids, carbohydrate, saponin and cardiac glycosides. The result showed that the *Costus afer* leaf methanol extract and snail slime were non-toxic to mice in sub-acute and acute dose of 5000 mg/kg. The blood glucose lowering effect was investigated for *C. afer* and snail slime at grade doses of 100mg/kg and 300mg/kg body weight of the rat for 21 days oral administration. There was blood glucose reduction for all doses of *C. afer*, Snail slime and the standard hypoglycemic drug (Glibenclamide, 5 mg/kg) investigated. This result approves the reported mechanisms of "[7], [8], [19]" whereby some herbal anti-diabetic remedies reduces blood glucose levels are more or less similar to those of synthetic oral hypoglycemic drugs like metformin and sulfonylurea etc. Still to that, medicinal and pharmacological activities of medicinal plants are often attributed to the presence of the so called secondary plant metabolites "[10, [13]". Our preliminary findings may further lend support that the snail slime in both acid and alkaline medium which proves slightly

soluble, may go a long way to act as a carrier of chemical and biological materials for use as nanoparticles in medical and pharmaceutical formulated drugs.

1. Introduction

Being that world population with diabetes is rising each year, therefore research on diabetic treatment is gaining more ground, this rise is expected to hit 439 million by 2030 "[18]". The knowledge of rise in population of diabetic patient has led to a vast discovery of new medications as well as natural products extracted from herbal plants. Many active ingredients extracted from herbal plants possess therapeutic values such as hypoglycemic activity, antioxidant action, etc. with many others yet to be discovered. The most studied and commonly used medicinal plants that its blood glucose lowering effects tested, studied and confirmed in different parts of the world include: *Allium cepa* (Onion), *Allium sativum* (Garlic), *Aloe vera, Cinnamomum tamala, Coccinia indica, Gymnema sylvestre* (Gurmar), *Murrayi koningii, Ocimum sanctum, Trigonella foenum-graecum* (Fenugreek), *Pterocarpus marsupium* (Indian Kino) *and Syzigium cumini* "[6], [8], [12]".

Diabetes Mellitus (DM) is one of the most widely occurring metabolic disorders throughout the world and it is characterized by chronic hyperglycemia as a result of insulin resistance or defect in insulin secretion or both. Death results from complications in some of these organs "[9], [14], [22]". It is a well-known fact that, several medicines are available for diabetic management, but they are associated with significant side effects which affects the quality of life. As a result of the aforementioned many herbal preparations has been carried out or conducted as an alternative in the role of diabetic management. Intense studies on herbal remedies will bring out a potentially powerful anti- diabetic therapy and will be immensely beneficial to patients.

Presently, type 2 diabetes mellitus, the most common type of diabetes mellitus, is managed by a combination of diet, exercise, oral hypoglycemic drugs and sometimes insulin injection "[5]". The main form of treatment of type 2 diabetes mellitus uses currently synthetic oral hypoglycemic drugs which have shown undesirable side effects and high secondary failure rates "[5]".

1.1. The Medicinal Plant (*Costus afer)*

Costus afer Ker Gawl. (Costaceae) is a tall perennial semi-woody herbaceous, unbranched medicinal plant with leafy canes, commonly found in moist or shady forests and riverbanks of tropical West Africa including Nigeria, Ghana and Cameroon "[15]". It may grow up to 3m high and belong to the family *Costaceae* "[11]". *Costus afer* bears terminal inflorescence of white and yellow flowers and is also commonly found in the forest zones of most places including Senegal, South Africa and Guinea and in the most region of tropical Africa, particularly in higher rainfall areas "[11], [20]". The plant is commonly called Ginger lily or Bush cane in English. In Igboland it is known as 'Okpete' or 'Okpoto' were as in Hausa and Yoruba it is called 'Kakizawa' and 'Tete-egun'"[4], [20]" respectively. It is as well called 'Mbritem' in Efik "[15]". Spiral Ginger is a common name given to plants of the family *Costaceae* which along with the family *Zingiberaceae* make up a 'super' family of plants generally known as the Gingers. The root extract is reported to have manifold uses such as genital stimulant, laxative and treatment of leprosy and stomach troubles, while the stem juice/sap has been used to treat arthritis, rheumatism and pharyngeal infections "[11]".

Figure 1. *Shows the Costus afer Ker Gawl. Leaf.*

1.2. Giant African Land Snails and Snail Slime

The group of snails commonly referred to as Giant African Land Snails belong to Phylum Mollusca and is used in this work to extract the snail slime. Slimes generally are any mucilaginous substance which exudes from the bodies of certain animals such as snails or slugs. The land snail (tropical snail) belongs to the class Gastropoda. Different species are abundant in Africa, Europe, India and in some other parts of the world. In Nigeria, it is called different names with respect to the geographical location. In Northern Nigeria, that is among the Hausa community it is called Dodon Kodi while in the Eastern Nigeria (Ibo) and Western Nigeria (Yoruba) it is called Ejula and Igbi respectively. Snail has a lot of slimy substances known as snail slime which it drops along its path as it moves. It also uses this slime to regenerate its shell and skin when damaged. The regenerative capacity of snail slime and the fact that diabetes is characterized by damage of the pancreatic beta cells, lead to this investigation for possible anti-diabetic activity. The people of West Africa have different preference for achatinids. In Nigeria, the species of choice is *Archachatina marginata* while in Ghana, the species of choice is Achatina (Achatina) achatina "[17]".

(a)

(b)

Figure 2. *(a) and (b) presents the picture of giant African land snail and the snail slime.*

2. Materials and Methods

2.1. Collection of Plant Materials and the Purchase of African Giant Land Snail

Costus afer was collected from Umuewi village in Njaba Local Government Area of Imo State. This was identified and confirmed by Mr. Cletus Ukwubile A., the Taxonomist, Biology Unit of Science Laboratory Technology of Federal Polytechnic Bali. The African giant land Snail was purchased from Afor Awo-Omamma Market of Oru – East Local Government Area of Imo State.

2.2. Animal Materials

Twenty four Swiss Albino Mice of both sexes weighing about (21 - 30g) and thirty five Swiss albino Rats were purchased from animal house, Department of Zoology University of Jos for toxicity and antidiabetic studies respectively. The animals were kept in a separate animal room on a 12 hours dark cycle at a room temperature of 27°C and with free access to food and water *ad libitum* for 4 days. The animals were fed standard rat pellets feed and filtered water.

2.3. Experimental Design

The rats that showed diabetic and healthy were randomly selected and distributed into 7 groups of 5 animals each. Animals in the different groups received either distilled water, left untreated, graded doses of the extract, snail slime and standard hypoglycemic drug (Glibenclamide).

Group A: - Normal Control (Distilled Water)

Group B: - Untreated Diabetic Control

Group C:- Costus afer Leaf Methanol Extract (CaLME) 100 mg/kg body weight

Group D:- Costus afer Leaf Methanol Extract (CaLME) 300 mg/kg body weight

Group E: - Snail Slime (SS) 100 mg/kg body weight

Group F: - Snail Slime (SS) 300 mg/kg body weight

Group G:-Standard hypoglycemic drug [Glibenclamide (GC)] 5 mg/kg

2.4. Drug Administration and Treatments

The solutions of the extracts were dissolved in distilled water according to the recommended graded doses for the experiment and given to the animals orally using an oral feeding needle (gavage). This was followed by monitoring the blood glucose level. The blood sample were collected at Day 0, Day 4, Day 7, Day 14 and Day 21 for all the groups of 5 animals by tail bleeding and the fasting blood glucose level was calculated by One Touch Glucometer which is expressed in mg/dL of blood.

3. Results

3.1. Solubility Investigation of Snail Slime

The solubility investigation of Snail slime was carried out using solvents like Distilled water, n-hexane, petroleum ether, methanol, ethanol and acetone; mineral acids like concentrated and dilute H_2SO_4 and HCl as well as bases like NaOH, and aqueous ammonia. This was conducted at different temperature ranges between 33°C and 45°C. The results of this investigation were shown on table 1

Table 1. *Solubility test of the snail slime at temperature ranging from 33°C -45°C.*

S/NO	TEST	OBSERVATION	INFERENCE
1.	Distilled water and the slime extract	PS	MS
2.	n-hexane and the slime extract	NS	NS
3.	Petroleum-ether and the slime extract	NS	NS
4.	Methanol and the slime extract	NS	NS
5.	Ethanol and the slime extract	NS	NS
6.	Acetone and the slime extract	NS	NS
7.	Aqueous ammonia and the slime extract	NS	NS
8.	1M of Dil. HCl and the slime extract	NS	NS
9.	Dil. Sulphuric Acid and the slime extract	NS	NS
10.	Conc. HCl and the slime extract	S	PS
11.	Conc. Sulphuric acid and the slime extract	LS	SS
12.	Conc. Ammonia and the slime extract	LS	SS
13.	Dil. NaOH and the slime extract	S	MS

PS=Partially soluble, MS=moderately soluble, NS=Not soluble, SS= sparingly soluble, LS=Less soluble, S=soluble

3.2. Investigation of the Physicochemical Properties of the Snail Slime Extract

Some Preliminary physicochemical properties indicating preliminary chemical analysis of the Snail slime was carried out using standard procedures adopted by "[1]".

Table 2. *Shows the Physicochemical analysis of the Snail slime.*

S/NO	TEST	OBSERVATION	INFERENCE
1.	5% NaOH was added to the distilled water with slime extract and 1% CuSO4 to the test tube.	Pink colour observed	Protein detected +++
2.	i) 2 drops of Conc. Nitric Acid added with distilled water and slime extract in a test tube and heated. ii) The solution was added with 3drops of NaOH.	i) White precipitate formed, on heating turns to yellow colour. ii) The solution changed to orange colour	Protein detected+++
3.	3 drops of Acetone with slime extract dropped on a filter paper.	Did not forms a translucent in the filter paper	Fat not detected +
4.	Acetone with slime extract was added with Fehling solution A and B Respectively.	Form a brown colour while in excess turns to purple colour.	Carbohydrate detected++
5.	Dis. Water added with slime extract and 1% of iodine was put into the test tube.	Form red-brick colour.	Carbohydrate detected++
6.	Conc. Sulphuric Acid added to the 1-naphthol in a test tube containing 2ml of the extract and distilled water.	Solution forms 3 phases of precipitate, red-brick and light-green colour.	Carbohydrate detected++
7.	5% of NaOH was added to 1ml of the extract and Ammonia chloride in a test tube.	White precipitate formed, on heating changed to light purple colour.	Carbohydrate detected++

3.3. Acute Toxicity Study of the *C. afer* Leaf Using Mice

The toxicology of the mice was carried out according to the dose provided by "[16]". It starting with 10 mg/kg, 100 mg/kg, and 1000 mg/kg for phase I while 1600 mg/kg, 2900 mg/kg and 5000 mg/kg was used for phase II acute toxicity determination. During this investigation the physical appearance and gross behavioral changes as well as the lethal dose effect were shown on Table 3 and 4. Furthermore the changes recorded in the body weight of the Swiss albino mice was shown on Table 5 and 6.

Table 3. *Shows the Observed Changes in Physical Appearance and Gross Behavioral Changes in Swiss Albino Mice in the 0 - 4 hrs, 24 h and 48 h of the Acute Toxicology Investigation.*

S/N	OBSERVATION	24 HRS					
		PE	SS	PE	SS	PE	SS
1	Hair	N	N	N	N	N	N
2	Eyes	N	N	N	N	N	N
3	Mucus membrane	N	N	N	N	N	N
4	Salivation	N	N	N	N	N	N
5	Sleep	N	N	N	N	N	N
6	Coma	N	N	N	N	N	N
7	Tremor	N	N	N	N	N	N
8	Diarrhea	N	N	N	N	N	N
9	Morbidity	N	N	N	N	N	N
10	Mortality	N	N	N	N	N	N

N=Normal, PE= Plant Extract (*Costus afer*), SS= Snail Slime

Table 4. *Acute Toxicity showing LD_{50} Effect of C. afer Leaf methanol Extract and the Snail slime Administered Intraperitoneally (i.p) to Swiss Albino Mice after 24 h.*

Experiment	Dose (mg/kg b.w)	Number of death Mice/Number of survived Mice after 24 h	Number of death of Treated mice/Number of survived Mice after 24 h
Phase 1(n=3)	10	0/3	0/3
	100	0/3	0/3
	1000	0/3	0/3
Phase II (n=1)	1600	0/1	0/1
	2900	0/1	0/1
	5000	0/1	0/1

Acute Toxicity Effect of C. afer Leaf methanol Extract and Snail Slime on the body weight of the Intraperitoneally Administered Swiss Albino Mice after Treatment.

Table 5. *Shows the Phase 1 Toxicity Test: Body weight determination.*

Body weight					
Group	**Treatment**	**Before Treatment**	**After Treatment**	**Calculated difference**	**Remark**
Mark at the ear	10 mg/kg b.w CaLME	23.50g	23.56g	0.06g	NS
Mark at the head	100 mg/kg b.w CaLME	27.43g	27.20g	0.23g	NS
Mark at the tail	1000 mg/kg b.w CaLME	28.33g	28.13g	0.20g	NS
Mark at the back	10 mg/kg b.w SS	22.57g	22.42g	0.15g	NS
Mark at the leg	100 mg/kg b.w SS	23.50g	23.05	0.45g	NS
Not Marked	1000 mg/kg b.w SS	29.68g	29.04g	0.64g	NS

CaLME = *Costus afer* leaf Methanol Extract, SS = Snail Slime, NS = Not Significant, B. W = Body Weight.

Table 6. *Shows the result of the Phase II Toxicity Test: Body weight Examination.*

Body weight					
Group	**Treatment**	**Before Treatment**	**After Treatment**	**Calculated difference**	**Remark**
Mark at the head	1600 mg/kg b.w CaLME	28.46 g	28.01 g	0.45 g	NS
Mark at the Leg	2900 mg/kg b.w CaLME	29.70 g	28.70 g	0.50 g	NS
Mark at the Back	5000 mg/kg b.w CaLME	29.88 g	29.34 g	0.54 g	NS
Mark at the Tail	1600 mg/kg b.w SS	29.74 g	29.12 g	0.62 g	NS
Mark at the Ear	2900 mg/kg b.w SS	29.86 g	29.13 g	0.73 g	NS
No Mark at the body	5000 mg/kg b.w SS	30.01 g	29.26 g	0.75 g	NS

CaLME = Costus afer Leaf Methanol Extract, SS = Snail Slime, NS = Not Significant, B. W = Body Weight.

3.4. Effect of *Costus afer* Leaf Methanol Extract and Snail Slime on Blood Glucose Level of Swiss Albino Rats

The summary result of the effect of the *C. afer* leaf methanol extract, the Snail slime and the standard hypoglycaemic drug (Glibenclamide, 5mg/kg) on blood glucose levels of the Swiss Albino Rats as well as the body weight calculated difference was recorded on day 0, day 4, day 7, day 14 and day 21 at different graded doses of 100 mg/kg and 300 mg/kg. The standard deviation, standard error of the mean and the number of animals per group were presented. These were shown on Table 7.

Table 7. *Indicates the summary of the result of the acute effect of C. afer leaf methanol extract and Snail Slime on blood glucose levels of the animals after oral daily administration for 21 days. Mean±S_D, S_M and n=5.*

Group	**Dose (mg/kg)**	**Body weight difference (g)**	**Blood glucose levels (mg/dL)**				
			Day 0	**Day 4**	**Day 7**	**Day 14**	**Day 21**
Normal Control		+0.28	109.6±8.32	108.8±5.81	111.2±2.86	110.4±6.27	114±6.52
			S_M = 3.72	2.60	1.28	2.80	2.94
Diabetic Control		-0.54	238±11.55	226±9.06	229.8±9.15	233±9.95	236.4±11.13
			S_M = 5.17	4.05	4.09	4.45	4.98
CaLME	100	-0.36	227.8±12.09	211.6±6.80	184.8±11.46	142.4±6.11	124.8±6.76
			S_M = 5.41	3.04	5.13	2.73	3.02
CaLME	300	-0.86	217±15.35	187.2±9.47	162.8±5.12	128±3.54	118±1.58
			S_M = 6.86	4.24	2.29	1.58	0.71
SS	100	-0.34	219.6±10.95	203.6±10.67	182.8±12.83	160±6.52	132.8±2.00
			S_M = 4.90	4.77	5.74	1.14	0.89
SS	300	-0.46	217.6±3.95	197.4±8.17	171.8±3.87	147.8±2.59	122.6±3.29
			S_M = 1.77	3.65	5.38	1.16	1.47
GC	5	-0.4	239.2±13.46	190±12.6	144.8±9.12	122.6±5.03	103.2±1.83
			S_M = 6.02	5.63	4.08	2.25	0.82

*S_D, S_M and n are the standard deviation, standard error of the mean and the number of the animals per group respectively.

4. Discussion

Investigation of the solubility profile indicates that the snail slime was partially soluble in distilled water at ordinary room temperature but moderately soluble at its boiling point. The snail slime was not soluble in n-hexane, petroleum ether, methanol, acetone, ethanol, aqueous ammonia, dilute and concentrated Hydrochloric acid, (HCl), Sulphuric acid (H_2SO_4), and Sodium Hydroxide (NaOH) at room temperature. There was a noticeable slight difference in its solubility enhancing effect at temperature of 33°C and 45°C.

The physicochemical determination carried out indicates that protein, carbohydrate and fat were present in variable and appreciable quantity. From the preliminary analysis of the physicochemical screening of the snail slime, there was indication that a high quantity of protein was present in the snail slime as compared to its carbohydrate and fat content.

The toxicology study of the mice was carried out in accordance with "[16]" method. The phase 1 dose was 10 mg/kg, 100 mg/kg, 1000 mg/kg. The phase II dose was 1600 mg/kg, 2900 mg/kg and 5000 mg/kg for one mouse each.

Few changes in physical appearance (morbidity) was observed after the 10 mg/kg b.w dose treatment and become pronounced at higher dose but reversed after 2 h of treatment. Acute toxicity study of *C. afer* and Snail Slime on mice shows that no mice died within 24 h as indicated on (Table 4) after intraperitoneal (i.p) administration with the extracts. The LD_{50} at 5000 mg/kg body weight approves the extracts as safe on the account of "[16]". The use of Lorke's method (LD_{50}) in determination of acute toxicity of the crude extract of *Costus afer* leaf was to investigate the levels of toxicity, that is, moderately toxic, slightly toxic, toxic or safe for human consumption when taken in diseased conditions "[21]". There was no death recorded among the dose groups for the two weeks experimental/investigation period which supports the claim. The result of the experiment performed reveals that the methanol extract of *Costus afer* was not toxic at any dose on the animal in the first 4 h, 24 h, 48 h, 7 days, and 21 days. Both at the lower (10 mg/kg b.w) and highest dose (5000 mg/kg b.w) there was no significant change observed in the calculated body weight and the behavioral parameters such as hair, eyes, mucous membrane, sleep, mortality etc. used to evaluate the toxicity. Earlier work of "[3]" approves that *Costus afer* leaf methanol extract and snail slime was nontoxic to mice in sub-acute and acute dose of 5000 mg/kg. However, it was observed that the calculated body weight of the mice did not significantly decreased after the intraperitoneally administration of the extracts. This indicates that the administration of the extract does not affect the growth of the mice.

The graded doses of 100 mg/kg and 300 mg/kg for snail slime and *C. afer leaf methanol* extract showed significant hypoglycemic effect on alloxan monohydrate induced diabetic rats. Oral administration of 5 mg/kg glibenclamide also produced significant reduction on induced diabetic rats than the *C. afer* leaf methanol extract and snail slime for the 21 days of investigation. On the other hand there were no signs of any side effect of the plant extract (*C. afer*) and snail slime on the animal model used. The absence of signs of any side effect is a major advantage the *Costus afer* leaf methanol extract and the Snail slime have over the standard hypoglycemic drug. During the long term (21 days) study of the effect of the plant extract and snail slime a physical parameter like the body weight was monitored and there was an indication of slight reduction or changes in the calculated body weight of the rats which was not significant.

From Table 7 there was an indication of reduction of the fasting blood glucose level from an average of 227.8 mg/dL for the low dose of 100 mg/kg body weight of the animal on treatment with *Costus afer* leaf methanol extract to 124.8 mg/dL after 21 days oral administration. There was no observable change indicated with regards to the body weight of the animals. The change in the calculated average body weight before the experiment and after the experiment was -0.36 g (that is a reduction in weight which was not significant after 21 days of oral administration of the extract).

Similarly, the effect of high dose of 300 mg/kg *Costus afer* leaf methanol extract on fasting blood glucose level for 21 days oral administration yielded an average reduction of 99 mg/dL(i.e. from 217 mg/dL to 118 mg/dL). The body weight reduction gave an average of -0.86 mg/dL which was not significant. However the high dose of the *Costus afer* methanol leaf extract on blood glucose level for 21 days oral administration shows more effect of hypoglycemia activity than the lower dose.

On the other hand, the effect of low dose of 100 mg/kg of snail slime (SS) on blood glucose level was investigated on five rats for 21 days oral administration. It indicated a reduction in blood glucose level from day 0 to day 21 with an average reduction of -86.8 mg/dL associated with non-significant calculated body weight reduction of -0.34 g. The effect for high dose of 300 mg/kg of snail slime on blood glucose level after 21 days of oral administration gave an average of -95 mg/dL blood glucose reduction and a non-significant calculated body weight reduction of -0.46 g. In all the calculation, the presence of minus (-) sign indicates the reduction obtained.

5. Conclusions

The result obtained shows that the *Costus afer* leaf methanol extract and Snail slime are non-toxic in sub-acute and acute dose of 5000 mg/kg. This study has joined the views of other researchers to claim that anti-diabetic medicinal plants do exert their blood glucose lowering effect by stimulating insulin secretion from pancreatic beta-cells, enhancing glucose uptake by fat and muscle cells, altering the activity of some enzymes that are involved in glucose metabolism or slowing down the absorption of sugars from the gut. Being that the snail slime in both acid and alkaline medium was slightly soluble, it may go a long way to act as a carrier of chemical and biological materials for use as nanoparticles in medical and pharmaceutical industry as it may possess the ability to release the drug intermittently. Most importantly, since Snail uses its slime to regenerate its shell and skin when damaged hence the regenerative capacity of snail slime and the fact that diabetes is characterized by damage of the pancreatic beta cells, may give credit to the hypoglycemic effect observed in *C. afer* methanol leaf extract and snail slime for possible drug formulation for anti-diabetic remedy.

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References

[1] Adikwu, M. U. and Ikejiuba C. C. (2005). Some physiological and wound healing properties of Snail mucin. Boll. Chim. Farm. 144:1-8.

[2] Adikwu, M. U. and Nnamani P. O. (2005). Some physiological and toxicological properties of Snail mucin. *Bioresearch* 5:1-6

[3] Agu, M. O., Barminas J. T., Ukwubile C. A., Hotton J. A (2015). Acute toxicity investigation of methanol leaf extract of Costus afer and Snail slime extract on Swiss albino Mice. American Journal of Chemistry and Application. vol. 2, No.6, pp 141-147.

[4] Anaga, A. O, Njoku C. J. Ekejiuba E. S. E siaka M. N. Asuzu I. U. (2004). Investigation of the methanolic leaf extract of *Costus afer* Ker for Pharmacological activities *in vitro* and *in vivo*. *Phytomedicine* (2-3): 242-248.

[5] Bailey, C. J., (2000). Potential new treatments for type 2 diabetes. *Trends in pharmacological sciences*. 21 (7): 259-265.

[6] Bailey, C. J., Day, C., (1989). Traditional plant medicine as treatments for diabetes. *Diabetes Care* 12: 553-564.

[7] Bastaki, S., (2005). Diabetes mellitus and its treatment. *Int. J. Diabetes & Metabolism* 13: 111- 134

[8] Bnouham, M., Ziyyat, A., Mekhfi, H., Tahri, A., and Legssyer, A., (2006). Medicinal plants with potential antidiabetic activity- A review of ten years of herbal medicine research (1990-2000), *International Journal of Diabetes & Metabolism* 14:1-25.

[9] Brownlee, M., (2001). Biochemistry and molecular biology of diabetic complications. *Nature* 414: 813-820.

[10] Bruneton, J., (1999). Pharmacognosy, Phytochemistry and Medicinal plants. Intercept. Ltd. England, U. K.

[11] Bukil, H. M. (1985). The useful plants of West Tropical Africa. Vol. 1 Royal botanic gardens, 63-67

[12] Grover, J. K., Yadav, S., and Vat, V., (2002). Medicinal plants of India with antidiabetic potential. *Journal of Ethnopharmacology* 81:81-90.

[13] Heinrich, M., Barnes, J., Gibbons, S., and Williamson, E. M., (2004). Fundamentals of Pharmacognosy and Phytotherapy. Churchill Livingstone, Elsevier Science Ltd., UK.

[14] Hirch, I. B., (1995). Glycemic control and complications of diabetes mellitus, *West Journal of Medicine.* 162(5): 430-438.

[15] Iwu, M. M., (1983) Traditional Igbo Medicine, Institute of African Studies publication. University of Nigeria, Nsukka pp112-144.

[16] Lorke, D. (1983). A new Approach to Practical Acute Toxicity Testing. *Arch. Toxicol*; 53: 275-289.

[17] Monney, K., (1994). Notable notes on Giant African Snails. *Snail Farming Research*. 5: 1-13.

[18] Shaw, J. E., Sicree R. A., Zimmet P. Z., (2010). Global estimates of the prevalence of diabetes.

[19] Tanira, M. O. M., (1994). Antidiabetic medicinal plants: a review of the present status and future directions, *Int. J. of Diabetes* 2(1): 15-22.

[20] Ukpabi, C. F. Agbafor K. N. Ndukwe O. K., Agwu A., Nwachukwu S. N. (2012). Phytochemical composition of *Costus afer* extract its alleviation of carbon tetrachloride induced hepatic oxidative stress and toxicity. *Int. J. of Modern Botany*. 2(5): 120-126.

[21] Ukwubile Cletus A., Agu Matthew O., Elsie Joseph A. (2015). Phytochemical screening and acute toxicity studies of *Melastomastrum capitatum* (Vahl) A. Fern and R. Fern (Melastomataceae) Leaf methanol extract. *American Journal of Biological Chemistry.* Vol.3 No. 4 pp 57-62.

[22] Weiss, J., and Sumpio, B., (2006). "Review of prevalence and outcome of vascular disease in patients with diabetes mellitus". *Eur J, Vasc. Endovasc. Surg.* 31 (2): 143-150.