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Extraction, Phytochemical, Physicochemical and Toxicological Study of Ginger Lily (*Costusafer* Ker Gawl) Leaf and Snail (*Archachatinamarginata*) Slime for Potential Use as Anti-Diabetic Drug Delivery.

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Abstract: *The leaves of Costusafer Ker Gawl., belongs to the family of Costaceae and has manifold uses in Eastern part of Nigeria, some other African countries as well as other parts of the world. Special part of this usefulness is as folk medicine in Eastern part of Nigeria for the treatment and management of a variety of human ailment, like stomach ache, swollen legs, diabetes mellitus etc. From the sequential extraction experiment conducted on Costusafer using Petroleum ether, Methanol, Acetone and water,the bioactive components was determined as well as the percentage yield. The result obtained for percentage yield of petroleum ether, methanol, acetone and water were 0.97, 6.32, 2.07 and 2.63 respectively. Investigation of the solubility profile of the snail slime indicated that it 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. From the physicochemical analysis of the snail slime it was observed that a high quantity of protein was present as compared to its carbohydrate and fat content. The preliminary phytochemical screening of methanol, acetone, and water extracts of the Costusafer leaves was conducted with indication of the presence of alkaloid, phenol, flavonoid and cardenoloids in the methanol extract; carbohydrate and saponin were present in the acetone extract; carbohydrate, glycosides, cardiac glycosides and saponin were present in the aqueous extract. The result obtained from the toxicology determination showed that the Costusafer leaf methanol extract and snail slime were non-toxic to mice in sub-acute and acute dose of 5000 mg/kg. The Costusafer leaf methanol extract was investigated for its effect on fasting blood glucose levels as well as body weight in normal and alloxan induced diabetic Swiss albino rats. The investigation extended to the determination of the long term (chronic) effects of Costusafer leaf crude methanol extract and that of snail slime on blood glucose levels of alloxan induced diabetic Swiss albino rats treated orally for 30 days on dose of 100 mg/kg body weight of the extracts. Fasting blood glucose levels and changes in body weight were evaluated on day 0, 7, 14, 21 and day 30 using Accu-Chek active Glucometer and electronic precision weighing balance respectively. 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.*

Keywords: *Phytomedicine, Synthetic drug, Costusafer, Snail Slime, Toxicology, Phytochemical*

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I. Introduction

The plant, *Costusafer* Ker Gawl, is a medicinal plant of the family of *Zingiberaceae* now known as*Costaceae.* It's an erect plant growing up to 3 meters tall, having stem horizontally striped at base; leaves narrowly lanceolate, dark green above and lighter green below (Bukil, 1985). Many plants especially*Costusafer* have both been reported traditionally to have different biochemical properties and also through phytochemical examination to possess anti- diabetic properties (Jayasari, 2009). This plant is widely distributed both in the Western, Eastern and Southern regions of Nigeria. The plant has various names but are commonly and popularly called Bush cane or Ginger lily in English. In Nigeria, the Igbo speaking tribe call it *"Okpoto"* or *"Okpete".*Towards the middle belt - the Hausa people of Northern Nigeria and the Yoruba of Western Nigeria call it *"Kakizawa"* and *"Tete-egun"* respectively.The Efik people call it "Mbritem" while the Cameroon (Anglophone) calls it "Monkey sugar cane"(Anaga *et al.*, 2004; van Wyk*et al.*, 1997 and Iwu, 1983).

The group of snails commonly referred to as Giant African Land Snails belong to Phylum Mollusca (Bequaert, 1950). 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 West and Central Africa, species of*Achatina* are confined to humid areas, as such majority of them are naturally confined to forested areas, while species of *Archachatina* are distributed in less humid areas (Raut and Barker, 2002). The land snail harbor and survive in most humid terrestrial habitat during the rainy season from March to October.

Different names were given to snails in Nigeria with respect to the geographical location they are found. In the Northern region, that is, among the Hausa community it is known as *DodonKod*i while in the Eastern region, that is, Igbo land and Western region, that is, Yoruba ethnic group it is called *Ejula* and *Igbin* respectively.

The snail exudes from its body a lot of slimy mucilaginous substance that is moist, soft and adhesive known as snail slime. Snails drops it along its path as it moves. The slime is beneficial to the snail as it uses it to regenerate its shell and skin when damaged.

There is a growing interest worldwide in phytomedicine because of their effectiveness, fewer side effects and low costs. This wisdom of traditional knowledge of medicinal plantsnecessitated my interest in the study of *Costusafer* for possible use as a potential for anti-diabetic treatment. Balde, *et al*., 2006 and Jung *et al*., 2006 bothreported separately about the role of medicinal plant in the management of diabetes mellitus as compared to the use of conventional anti-diabetic therapies which many users do not have access to in the developing countries as well as the use of anti-diabetic herbal remedies which is declining in developed countries since the introduction of synthetic oral hypoglycaemic agents and insulin.

Generally, interest has been renewed in the use of medicinal plants with hypoglycaemic potential which is believed to be motivated by several factors, like, the side effects, high secondary failure rates and the high cost of drugs attributed to the use of the synthetic oral hypoglycaemic drug (Bailey, 2000).

The World Health Organization (WHO, 2002) has also realized that an effective health agenda for developing countries can never be achieved by western medicine alone, unless it is complemented by alternative medicines including traditional herbal medicine and has accordingly advised and urged developing countries of the world to utilize their medicinal plant resources and other traditional medicine systems in order to achieve the goal of primary health care (WHO, 2002).

Based on the aforementioned, it is reported that in developed countries, sufferers of chronic diseases are turning to herbal remedies as alternatives to modern synthetic drugs (Calixto, 2000). Apart from the world-wide economic crunch, there is renewed interest in the use of herbal medicine in developed and developing countries which is motivated by some factors which include:

i.) High cost of synthetic drugs: Herbal medicines are usually less expensive than synthetic drugs.
ii.) Side effects of modern drugs: Although synthetic drugs show greater or faster effects than the equivalent phytomedicine, they also present a high degree of side effects and risks (Aronson, 2009; Haq, 2004).
iii.) The effectiveness of plant remedies: According to Iwu*et al.*, (1999), medicinal plants are gentle, effective and often specific in function to organs or systems of the body.

The aim of the study is to generally investigate the anti-diabetic potentials of bioactive crude of *Costusafer* Ker Gawl methanol leafextract, giant African land snail slime and their combination through the following objectives which are to:

(i). Extract some bioactive components from the leaf of *Costusafer*Ker Gawlwith solvents of different polarity such as petroleum ether, acetone, methanol and water.
(ii). Determine the solubility profile of the snail slime extract with mineral acids and solvents like acetone, ethanol, water, sodium hydroxide and hydrochloric acid.
(iii). Determine the preliminary physicochemical analysis of snail slime (i.e. the presence of carbohydrate, protein, sugar, fats and oils).
(iv). Qualitatively identify the class(es) of secondary plant metabolites present in the crude active *C. afer* leaf extract(s) by means of standard phytochemical screening tests.
(v). Determine acute toxicity profile of*Costusafer* and the Snail slime in a normal and alloxan- induced diabetic white Albino mice.

II. Materials and Methods

2.1 Collection of Plant Materials

*Costusafer*Ker Gawlplants leaves were obtained fromOhiaImeOrieUmuewi Village in Njaba L.G.A. of Imo State. The plant was carefully protected and parcelled to avoid the leaves drying abnormally and against laboratory practice. It was subsequently transported to Bali, Taraba State. The plant was identified and authenticated by Mr. Cletus Ukwubile A., the taxonomist, Biology Unit of Science Laboratory Technology of Federal Polytechnic Bali.

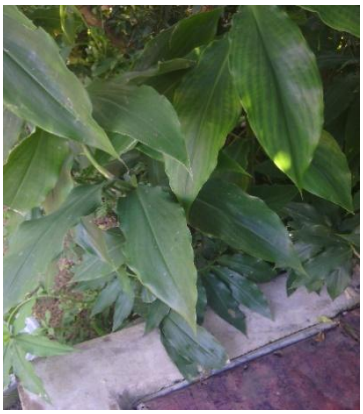

Plate 1: Costusafer plant

2.2 Purchase of Giant African Land Snails *(Archachatinamarginata)*

The giant African land Snails was purchased from Eke Okwudor market in Njaba L.G.A. of Imo State. They were carefully packaged in a cage and transported to Bali, Taraba State, the experimental/research point.

2.3 Plant Processing, Extraction, Phytochemical screening,Physicochemical and Toxicological study and Anti-diabetic effects of both Plant and Animal Materials on Rats.

2.3.1 Successive Extraction of *Costusafer* leaves

On arrival to the experimental/research point the leaves were separated from the stem and the rotten leaves were discarded accordingly. The plant leaves were washed, air dried under room temperature on the laboratory tables and pulverized using mechanical Corona hand grinder. To obtain the bioactive extracts of petroleum ether, acetone, methanol and water from the plant leaves a successive extraction method was used. Three hundred (300 grams) powdered leaf samples were extracted using 1200 mL each of hexane, acetone, methanol and water using cold maceration technique.The residue was removed by filtration, the filtrate was concentrated, that is, distilled, evaporated and vacuum dried under reduced pressure using rotary evaporator at 40^{o}C.

2.3.2 Extraction of Snail Slime from Giant African Land Snail

When the Giant African Land Snail got to the research venue it was allowed to acclimatize for two weeks. That is, the Snail was allowed to move freely and fed appropriately in a confined environment for two week to remove every shock it sustained from the time of purchase and transportation to the research venue. Themethod of Adikwu and Nnamani (2005) was employed for the preparation and extraction of snail slime from the Giant African Land Snail. The Giant African Land Snails (*Achachatinamarginata*) were washed with clean water to remove dirt and dust particles on the shells. The shells were knocked open at the apex. The inner content (i.e. fleshy body) of the snails was separated from the shells by a mechanical means involving the use of a spirally coiled rod inserted to remove the fleshy body. The mucus layer (slime) was gently scrapped off from the fleshy parts, pooled together in a container and precipitated with chilled acetone. The precipitates was freeze dried to obtain greyish – brown flakes of the snail slime extract, which was then grounded into fine powder, packaged and stored in a refrigerator until the time for the examination or studies.

a b

Plate11: (a) Giant African Land Snails and (b) Trails of Snail Slime as produced by the Snails

2.3.3 Preliminary Phytochemical Screening Analysis of C. afer

The bioactive leaf extract of *Costusafer*Ker Gawl. was subjected to the following Phytochemical test for the identification of its various bioactive constituents by standard methods of Evans (2006).

Test for Alkaloids

About 0.5 g of the bioactive component of *C.afer* methanol leaf extract was warmed with 10 mL of 2 % H_2SO_4 for 2 min and filtered. Three - one mL portions were treated with a few drops of Dragendoff's reagent, Wagner's reagent and Mayer's reagent respectively. Orange and red precipitate was seen with Dragendoff's and Wagner's solution respectively while a white to bluff precipitate was observed with Mayer's reagent.

Test for Saponins

About 0.5 g of the bioactive component of *C.afer*methanol leaf extract was shaken with 5 mL distilled water and heated toboiling point. Frothing shows the presence of saponins. The filtrate were added to 3 mL ofparaffin oil and thoroughly shaken to forma stable emulsion. This was left to stand for about 5 minutes. The presence of a stable emulsion indicates the presence of saponins.

Test for Tannins

About 0.5 g of the bioactive component of *C.afer* methanol leaf extract was mixed with water and heated on a water bath. The mixture was filtered and ferric chloride reagent was added to the filtrate. A dark blue or dark green solution indicates the presence of tannins. For hydrolysable tannins, shake 4 mL of extract with 4 mL of ammonia solution. Formation of an emulsion on shaking shows the presence of hydrolysable tannins.

Test for Glycosides

(a) The aqueous extract of the bioactive component of *C.afer* methanol leaf extract was boiled with a drop of Fehling's solution A and Fehling's solution B for 2 minutes. The presence of reducing sugar is indicated by an orange red precipitate on boiling with the Fehling's solution.

b) The extract is hydrolyzed with dilute Hydrochloric acid (HCl) solution and neutralized with sodium hydroxide (NaOH) solution. A drop of Fehling's solution A and B were then added and the presence of further red precipitate indicates the presence of glycosides.

Test for Phlobatanins

An amount of 0.5 mLof the filtrate was boiled with 5 mL of 1 % HCl (hydrochloric acid). A red precipitate shows presence of Phlobatanins.

Tests for Carbohydrate

Molisch Test

To a small portion of the*C.afer* methanol leaf extract in a test tube, few drops of molisch reagent was added and concentratedsulphuric acid (H_2SO_4) was added down the side of the test tube to form a lower, reddish coloring at the interphase which indicates presence of carbohydrates.

Fehling Test

An amount of 5 mL of an equal mixture of Fehling solution of A and B was added to a small portion of the *C.afer* methanol leaf extract in a test tube, and was boiled on a water bath, brick red precipitate indicate presence of reducing sugar.

Test for Flavonoids

An amount of1 mL of the *C.afer* methanol leaf extract was dissolved in dilute Sodium hydroxide solution. A yellow solution that turn colourless on addition of hydrochloric acid (HCl) shows the presence of Flavonoids.

2.3.4 Solubility profile of the Snail Slime extract.

The solubility of snail slime extract in different solvents was determined by dispersing a definite quantity (100 mg) of the snail slime extract in definite volume of each solvent- acetone, alcohol (ethanol), water, 0.1 M Sodium hydroxide, 0.1 M Hydrochloric acid and 0.1 M Ammonia hydroxide at different temperatures (15, 28, 36, 40^{o}C).

2.3.5 Determination of the physicochemical properties of the Snail Slime extract

Some physicochemical properties showing some preliminary physical and chemical analysis was carried out using standard procedures of (Adikwu and Ikejiuba, 2005).

Test for Proteins (Amines, Oxidation test)

Biuret test

Two drops of water and 1 mL of dilute sodium hydroxide were added to 2 % dispersion of snail slime extract in water. Two drops of 1 % Copper sulphate solution were added with the solution shaken thoroughly after each drop and observed. A purple or pink colour shows the presence of protein.

Xanthoproteic reaction

Two drops of concentrated Nitric acid were carefully added to 2 % dispersion of snail slime extract in water. A white precipitate was formed, which turned yellow on heating. The content of the test tube were cooled, three drops of dilute sodium hydroxide solution added and the precipitate observed. A yellow colour which changes to orange indicates the presence of proteins.

Test for fats and oils

A drop of the acetone extract of the snail slime was placed on a filter paper. The solvent was allowed to evaporate and the filter paper observed carefully for any translucence to indicate the presence of fats

Test for sugar

Three drops of freshly prepared Fehling's solution I and II were added to 1 % w/v aqueous dispersion of snail slime which was then heated in a boiling water bath for five (5) minutes and brown colour observed while in excess purple to indicate presence of carbohydrate.

Test for carbohydrates

Iodine test

Two drops of 1 % iodine solution were added to 1 mL of 1 % w/v of snail slime extract and then observed for blue black colouration which indicated the presence of carbohydrate.

Molisch's test

Two drops of α- naphthol solution was added to 2 mL of the snail slime extract dispersion and the two mixed thoroughly. Then 1 mL of concentrated Sulphuric acid (H_2SO_4) was gently poured down the side of the tube and red, brick and light green colour observed was an indication of the presence of carbohydrate.

Tollen's reagent test.

Tollen's reagent prepared as 1 mL of 5 % silver nitrate solution was treated with a few drops of 5 % sodium hydroxide solution. A volume of aqueous Ammonia just enough to re-dissolve the precipitate was added to 3 drops of the snail slime extract dispersion and the mixture warmed in a boiling water bath for few minutes. The colour of the precipitate formed was observed as white precipitate formed on heating and changed to light purple colour. This indicated the presence of carbohydrate.

2.3.6Toxicology Study of *C. afer*methanol leaf extract and Snail Slime.

An amount of0.5g of both the crude extract of *C.afer* leaves and snail slime was weighed individually and prepared into appropriate mililiters in order to determine the lethal dose (LD_{50}) (i.e. acute toxicity) in Swiss white albino mice. The crude extract of *Costusafer* leaves and snail slime was separately evaluated for their toxicity in non – infected albino mice aged 4 weeks and weighing 20-27 g using Lorke (1983) method of determining toxicity level of extract in mice. The test was carried out in two phases. In the phase I of the study,nine albino mice were randomized into three groups of three mice each. They were given 10 mg/kg, 100mg/kg and 1000mg/kg body weight respectively of the extract from *C.afer* and Snail slime administered orally. The albino mice were observed for changes in physical appearance, gross behavioural change and death in the first 0 h - 72 h and subsequently daily for 30 days.

In view of the result obtained from phase 1 treatment, phase II treatment was carried out using another fresh set of three albino mice randomized into three groups of one mice each and given 1600 mg/kg, 2900 mg/kg and 5000mg/kg body weight of the extract. These were observed for signsof toxicity and mortality for the first 0 h - 72 h and thereafter for 30 days.

2.3.7 Animals for the study

Albino rats of equal but different sexes with body weight ranging from (140 - 220g) were obtained from University of Jos, Nigeria and used for the study. The animals were maintained in a laboratory temperature between 28^{o}C to 32^{o}C. The animals were fed with standard rat pellets feed and filtered water *ad libitum*. The animals were kept in individual cages in anenvironmentally controlled room with a 12 h light/12 h dark cycle. Animals described as fasted were deprived of food for 24 h but allowed free access to water.

2.3.8 Experimental Grouping of Animal

The experimental rats were divided into nine (5) groups of five (5) animals in each group. The rats that showed diabetic and healthy were randomly selected and distributed into 5 groups of 5 animals each. Animals in the different groups received either distilled water, left untreated, plant or animal dose of the extracts and standard hypoglycaemic drug (Glibenclamide). The extract was administered for a period of 30 days (4 weeks). Body weights of the animals were recorded every week.

2.3.9 Experimental Designfor Diabetic Administration

The rats that showed diabetic and healthy were randomly selected and distributed into 5 groups of 5 animals each. Animals in the different groups received either distilled water, left untreated, plant or animal doses of the extracts and standard hypoglycaemic drug (Glibenclamide).

Group A: - Normal Control (Distilled Water)
Group B: - Untreated Diabetic Control
Group C: - Costusafer Leaf Extract (CaLE) 100 mg/kg body weight
Group D: - Snail Slime Extract (SSE) 100 mg/kg body weight
GroupE: - Standard hypoglycaemic drug [Glibenclamide (GC)] 5 mg/kg

2.3.10 Drug administration and treatments

The solutions of the extracts were dissolved in distilled water according to the recommended doses for the experiment and given to the animals orally. This was followed by monitoring the blood glucose level. The blood samples were collected at Initial (Day 0), First week (Day 7), Second week (Day 14), Third week (Day 21) and Fourth week (Day 30) for all the groups of 5 animals by tail bleeding and the fasting blood glucose level was calculated by Accu – Chek (Active) Glucometer which is expressed in mg/dL of blood.

III. Results

3.1 Yield in Percent of the Plant Extract

The result of the successive extraction process conducted is as shown on Table1. The following expression was used to determine the percentage yield of the bioactive extract of *Costusafer*on solvents of different polarity.

$$\textbf{Yield in percent (Y\%)} = \frac{\textbf{Weight of pulverized sample } (W_p)}{\textbf{Weight of Bioactive extract } (W_b)} \times \frac{100}{1}$$

where, W_p = Weight of the pulverised sample
W_b = Weight of the Bioactive extract

Table 1: Shows the Yieldin percentage of the various solvents used for the successive extraction of the bioactive component of the plant material (C.afer).

S/No	Solvents used	Quantity of Pulverised sample (W_p)	Weight of Bioactive extract (W_b)	Yield (%)
i.	**Petroleum ether**	300	2.9	0.97
ii.	**Methanol**	300	18.96	6.32
iii.	**Acetone**	300	6.2	2.07
iv.	**Water**	300	7.88	2.63

W_p= Weight of pulverised sample, W_b = Weight of the bioactive extract, Y % = Yield in percent.

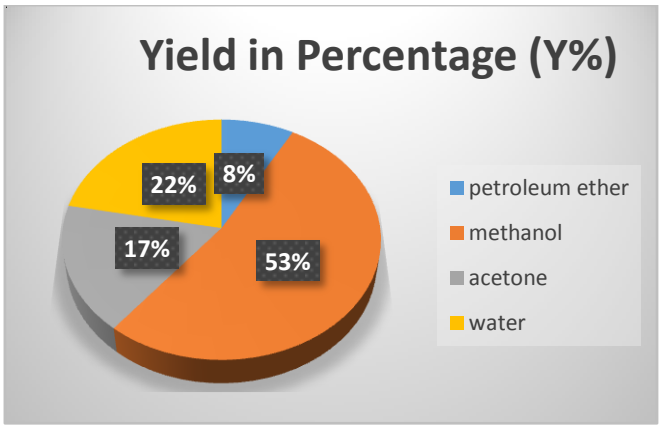

Figure 1: Pie chart representation of the bioactive extract of C.afer when extracted with petroleum ether, methanol, acetone and water.

3.2 Preliminary Phytochemical Screening of the Plant (*C.afer*) Extract

The preliminary phytochemical screening was conducted on the bioactive extract of *C.afer*leaves using the method adopted by (Evans, 2006). The results of the detected components were shown on Table 2.

Table 2: Presented the result of the Preliminary Phytochemical Screening of the bioactive components of C. afer leaf extract using Petroleum ether, Methanol, Acetone and Water.

S/No		Solvents used for the extraction			
	Plant Chemical Components	Petroleum ether	Methanol	Acetone	Water
a.	Alkaloids	-	++	-	-
b.	Saponins	-	+	++	++
c.	Tannins	-	+	-	-
d.	Glycosides	-	-	-	++
e.	Cardiac Glycosides	-	-	-	++
f.	Phlobatannins	-	-	-	-
g.	Carbohydrate	-	-	++	++
h.	Flavonoids	-	++	-	-
i.	Cardenolides	-	++	-	-
j.	Phenols	-	++	-	-
k.	Protein	-	+	+	+
l.	Oils	++	-	-	-

Keys: - Not detected; + Trace amount; ++ Moderately detected

3.3 Solubility Profile of Snail Slime Extract

The solubility test of the Snail Slime was conducted using some solvents like n-hexane, ethanol, methanol, water and acetone, mineral acids and bases. These were tested at different temperature ranging from 15^0C to 45^0C.

Investigation of the solubility profile indicated 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, and ethanol but partially/sparingly soluble in 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 33^0C and 45^0C. This high level of insolubility observed with snail slime at these temperature ranges could place snail slime as possessing some properties of choice as biological carrier for drug delivery vehicle.

3.3. Preliminary investigatory study of the physicochemical properties of the Snail Slime extract

The standard procedures adopted by Adikwuand Ikejiuba(2005) was used to obtain the results of some preliminary physicochemical properties of the snail slime extract presented below.

i. Protein Determination

a. An amount of 5 % NaOH was added to distilled water with snail slime extract and 1 % $CuSO_4$ to the tube and a Pink colour was observed indicating the presence of Protein in a high amount.

b. Alternatively, to determine the presence of protein in snail slime, 2 drops of Conc. Nitric Acid was added with distilled water and snail slime in a test tube and heated. There was a resultant white precipitate formed and further heating turned to yellow colour. An

ii. Fat Determination

An amount of 3 drops of Acetone with snail slime extract was dropped on a filter paper, there was no formation of a translucent on the filter paper which indicated the absence of Fat.

iii. Sugar Determination

Three drops of freshly prepared Fehling's solution I and II were added to 1 % w/v aqueous dispersion of snail slime which was then heated in a boiling water bath for five (5) minutes and brown colour observed while in excess purple to indicate presence of carbohydrate.

iv.Carbohydrate Determination

a. An amount of 3 mL Acetone was added to a quantity of snail slime and with Fehling solution A and B, a brown colour was observed which turned to purple in excess indicating the presence of carbohydrate.
b. Distilled water was added to the snail slime extract in a test tube followed by an additional 1 % of iodine, a red brick colour was obtained indicating the presence of Carbohydrate.
c. About 5 % of NaOH was added to 1 mL of the snail slime extract with Ammonium Chloride and a white precipitate formed which turned to light purple colour on heating, indicating the presence of Carbohydrate.

3.4 Acute toxicity study of the extracted bioactive components of the *C. afer*leaf and Snail Slime on mice.

The *C. afer*and Snail Slime toxicology study on mice was carried out according to the method adopted by Lorke (1983).

Table 3: Changes Observed on Body Appearance and Gross Behavioural Changes on Mice in 0 - 24 hr, 25-48hr and 49- 72hr of the Acute Toxicology Investigation.

S/N	Observation	0 - 24 hr		25 – 48 hr		49 - 72hr	
		C.aE	SE	C.aE	SE	C.aE	SE
1	Coma	NC	NC	NC	NC	NC	NC
2	Diarrhoea	NC	NC	NC	NC	NC	NC
3	Eyes	NC	NC	NC	NC	NC	NC
4	Hair	NC	NC	NC	NC	NC	NC
5	Morbidity	NC	NC	NC	NC	NC	NC
6	Mortality	NC	NC	NC	NC	NC	NC
7	Mucus	NC	NC	NC	NC	NC	NC
8	Salivation	NC	NC	NC	NC	NC	NC
9	Sleep	NC	NC	NC	NC	NC	NC
10	Tremor	NC	NC	NC	NC	NC	NC

NC = No Changes;C.aE = *C.afer* Extract;SE = Slime Extract,

Table 4: Acute toxicology investigation (LD_{50}) Effect of C.afer Leaf Methanol Extracts (Bioactive Components) and Snail Slime Administered Orally on Swiss Albino Mice after 0-72 hrs and within 30 days

Experiment	Dose (mg/kg b.w)	Number of death Mice/Number of survived Mice after 24 hrs, 48 hrs. and 72 hrs.	Number of death of Treated mice/Number of survived Mice after 24 hrs, 48 hrs.and 72 hrs.
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

Table 5: Acute Toxicity Effect of Methanol Extract of C. aferLeaf on the Body Weight of the Swiss Albino Mice after 30 DaysDrug treatment which wasOrallyAdministered.

Table 5a: Phase 1 Toxicity Test: Body Weight Determination.

Group	Treatment(mg/kg)	Body Weight(g) Before Drug Administration(b)	After Drug Administration(a)	Weight Gain(a-b)	Remark
A	10 mg/kg b.wC.aLE	24.36g	24.44g	0.08g	NS
B	100 mg/kg b.wC.aLE	26.71g	26.80g	0.09g	NS
C	1000 mg/kg b.wC.aLE	24.62g	24.74g	0.12g	NS

C.aE = *Costusafer*Leaf Extract, NS = Non Significant, b.w = body Weight, a= Final body weight, b = Initial body weight

Table 5: *Acute Toxicity Effect of Methanol Extract of C. aferLeaf on the Body Weight of the Swiss Albino Mice after 30 DaysDrug treatment which wasOrallyAdministered.*

Table 5b: *Phase II Toxicity Test: Body Weight Determination.*

Group	Treatment(mg/kg)	Body Weight (g) Before Drug Administration(b)	After Drug Administration(a)	Weight gain(a-b)	Remark
U	1600 mg/kg b.wC.aLE	25.32	25.58	0.26	NS
V	2900 mg/kg b.wC.aLE	22.50	22.82	0.32	NS
W	5000 mg/kg b.wC.aLE	26.37	26.85	0.48	NS

C.aE = *Costusafer*Leaf Extract, NS = Non Significant, b.w = body Weight, a= Final body weight, b = Initial body weight

Table 6: *Acute Toxicity Effect of Snail Slime on the Body Weight of the Swiss Albino Mice after 30 DaysDrug treatment which was OrallyAdministered*

Table 6a: *Phase 1 Toxicity Test: Body Weight Determination.*

Group	Treatment(mg/kg)	Body Weight(g) Before Drug Administration(b)	After Drug Administration(a)	Weight Gain(a-b)	Remark
A	10 mg/kg b.w SE	22.53g	22.69g	0.16g	NS
B	100 mg/kg b.w SE	23.85g	24.04	0.19g	NS
C	1000 mg/kg b.w SE	21.64g	21.85g	0.21g	NS

SE = Extract, NS = Non Significant, b.w = body Weight, a= Final body weight, b = Initial body weight

Table 6: Acute Toxicity Effect of Snail Slime on the Body Weight of the Swiss Albino Mice after 30 DaysDrug treatment which was OrallyAdministered

Table 6b: *Phase 11 Toxicity Test: Body Weight Determination.*

Group	Treatment(mg/kg)	Body Weight(g) Before Drug Administration(b)	After Drug Administration(a)	Weight Gain(a-b)	Remark
X	1600 mg/kg b.w SE	25.96	26.47	0.51	NS
Y	2900 mg/kg b.w SE	24.82	25.28	0.46	NS
Z	5000 mg/kg b.w SE	23.58	23.96	0.38	NS

SE = Extract, NS = Non Significant, b.w = body Weight, a= Final body weight, b = Initial body weight

Table 7: *Physicochemical Analysis of the Snail Slime Extract*

S/No	Physicochemical Properties	Test	Observation	Result
1.	Protein (Biuret test)	5% NaOH was added to the distilled water with slime extract and 1% $CuSO_4$ to the test tube.	Pink colour observed	+++
2.	Protein (Xanthoproteic reaction)	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	+++
3.	Fats and Oils	3 drops of Acetone with slime extract dropped on a filter paper.	Did not form a translucent in the filter paper	+
4.	Test for Sugar	Acetone with slime extract was added with Fehling solution A and B respectively.	Form a brown colour while in excess turns to purple colour.	++
5.	Carbohydrates	Distilled Water added with slime extract and 1% of iodine was put into the test tube.	Form red-brick colour.	++
6.	Molisch's Test	Conc. Sulphuric acid added to 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.	++
7.	Tollen's reagent Test	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.	++

+ = trace amount, ++ = moderately detected, +++ = detected at high amount,

Table 8: *Effect of the dose (100 mg/kg) of the Oral Administered Extract (C. afer, Snail Slime) on Blood Glucose Level of Rats after Daily Administration for 30 days*

Days	Dose mg/kg	Normal control	Diabetic Control	CaLE (100mg/kg)	SSE (100mg/kg)	GC (5mg/kg)
Day 0	100	126.2	225.3	207.5	218.5	224
Day 7	100	121.3	226.2	209	198.9	176
Day 14	100	124.4	224.7	174	174.6	154.7
Day 21	100	125.2	226.9	134	152	121.2
Day 30	100	123.5	223.5	119	120	105

CaLE=*Costusafer* LeafExtract, SSE= Snail Slime Extract,GC= Glibenclamide

Table 9: *Effect of the administered dose (100mg/kg) of the extracts (C. afer and Snail Slime) on the body weight of the Swiss Albino Rat after 30 days of treatment.*

Group	Dose (mg/kg)	Initial body weight (b)	Final body weight (a)	Difference in Body weight(g) = a-b
Normal Control		168.0	168.5	0.5
CaLE	100	172.6	172.9	0.3
SSE	100	195.4	195.8	0.4
GC	100	199.1	199.3	0.2

CaLE=*Costusafer* LeafExtract, SSE= Snail Slime Extract, GC= Glibenclamide

IV. Discussion

The bioactive components as obtained from the C.afer leaf using different solvents like Petroleum ether, acetone, methanol and water and the result percentage by yield was presented in Table 1. It was discovered that for every 300 g/L (w/v) of the plant material extracted with solvents such as petroleum ether, methanol, acetone and water a percentage yield of 0.97 %, 6.32 %, 2.07 % and 2.63 % respectively was obtained. There was an indication that methanol gave the highest yield (6.32 %) of the bioactive component from the plant extract which reflected with the result of the preliminary phytochemical screening of the Plant extract.

The Tables 2 above depicts the results of the preliminary phytochemical screening of the different solvents - petroleum ether, methanol, acetone and water used for the extraction. From the sequential extraction of the bioactive constituents of Costusafer leaf, the major constituents of the extract found during the phytochemical analysis were alkaloids, flavonoids, tannins, glycosides, saponins, proteins, cardenolides. The detected secondary metabolites from the four solvents used increases from petroleum ether, acetone, water and methanol. From the above study, it was observed that in the methanol extract more of the secondary metabolites were identified from the plant material used and it was responsible for the pharmacological activities seen in these investigations. According to Choi et al., (1991), many hundreds of plant extracts known and recorded possess flavonoids and are used for treatment of diabetes. Iwu (1983), mentioned that tannin-containing drugs demonstrated anti-diabetic activity. The presence of these phytochemicals mostly in the crude leaf extract is responsible for the regular use of the leaf by herbalist or traditional health practioneers in treating various diseases, as each of these constituents plays series of independent roles in biological activities of the plant (Ukwubile, et al., 2015). From the above records of the activity of the active principles of plants extracts, it may be possible that the insulin–like power of these bioactive compounds seen in Costusafer were responsible for its hypoglycemic effects.

From the physicochemical analysis of the snail slime extract, there was indication that a high quantity of protein was present while sugar and carbohydrate was determined on a moderate scale in the snail slime extract. The presence of fats and oils was on a trace level as compared to its carbohydrate and fat content as shown on Table 7.

With respect to the toxicology determination according to Lorke (1983) which commenced with 10 mg/kg, 100 mg/kg, and 1000 mg/kg for the first phase treatment while 1600 mg/kg, 2900 mg/kg and 5000 mg/kg for the second phase acute toxicity determination was shown on Tables 3, 4, 5 and 6 for observed body/behavioural changes, death and survival after 30 days,body weight determination for the plant material and snail slime respectively.

The phase 1 dose was 10mg/kg, 100mg/kg, 1000mg/kg for three mice in each treatment dose. The phase II dose was 1600mg/kg, 2900mg/kg and 5000mg/kg for one mouse each. Few changes in physical appearance (morbidity, hair and sleep) was observed after the 10mg/kg b.w dose treatment and become pronounced at higher dose but reversed after few hours of treatment. Acute toxicity study of C. aferand Snail Slime on mice shows that no mice died within 72 hours and for the 30 days of the treatment as indicated on (Table 4).As there was no death after oral administration with the extracts and the LD50 at 5000mg/kg body weight approves the extracts as safe on the account of Lorke (1983). The use of Lorke's method (LD50) in determination of acute toxicity of the crude extract of Costusafer 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 (Ukwubileet al., 2015). There was no death recorded among the dose groups for the two weeks experimental/investigation period which supports the claim that no death at dose rate of 10 mg/kg to 5000 mg/kg made the investigation to stand as non-toxic. The result of the experiment performed reveals that the methanol extract of Costusafer was not toxic at any dose on the animal in the first 0 - 72 h and 30 days. Both at the lowest(10mg/kg b.w) and highest dose (5000mg/kg b.w) no significant change was observed in the body weight and the behavioural parameters used to evaluate the toxicity for example, hair, eyes, mucous membrane, sleep, mortality etc. However, it was observed that the body weight of the mice slightly increased after the oral administration of the extracts which was not significant. This indicates that the administration of the extracts does not in any way affect the growth of the mice.

The dose of 100 mg/kg for snail slime, C.aferextract 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 extract, snail slime extracts for the 30 days of test. On the other hand there were no signs of any side effect of the plant extract (C.afer), snail slime extracts on the animals used which is in line with the result obtained from the toxicology determination. On the other hand, during the long term (30 days) in vivo study of the effect of the Costusaferextract, snail slime extracts, a physical parameter like the body weight was monitored and there was an indication of slight reduction in weight which was not significant.

V. Conclusions

This research work was designed to lead to the formation of new strategies for the development of novel antidiabetic drug to treat this serious condition (diabetes) which represents a global public health problem. This result approves the reported mechanisms of (Tanira, 1994; Bastaki, 2005; Bnouham*et al*., 2006) whereby some herbal anti-diabetic remedies which reduce blood glucose levels were 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 (Bruneton, 1999; Henrich*et al*., 2004). The result of the investigation of the plant's anti-diabetic activity has gained the support of the work of Tanira, 1994 which states 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. Ordinarily, it could be suggested that blood glucose lowering effect of antidiabetic medicinal plants through stimulating insulin secretion from pancreatic beta cells can be compared with the chemical activity that brought about Snail using its slime to regenerate its shell and skin when damaged.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*leaf methanol extract and snail slime extract for anti-diabetic remedy

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