

Trace mineral, qualitative phytochemical composition and antidiabetic effect of ethanol extract of *Citrullus lanatus* seeds on diabetic rats

ABSTRACT

The trace mineral concentration, phytochemical composition and antidiabetic effect of the ethanol extract of *C. lanatus* seeds were carried out using standard methods. The preclinical experimental model was 120mg/kg b.w. (via intraperitoneal) Alloxan induced diabetic rat model, with ethanol extract of *C. lanatus* seeds administered orally at 500mg/kg b.w. Qualitative phytochemical screening showed presence of alkaloids, flavonoids, saponins, cardiac glycosides, steroids/triterpenoids, tannins, carbohydrates and oils. Atomic Absorption Spectrophotometric analysis revealed that iron had the highest value (8.31mg/kg), followed by zinc (5.78mg/kg), then manganese (4.28mg/kg). Selenium concentration was appreciable (0.85mg/kg). Ethanol extract of *C. lanatus* seeds significantly ($p < 0.05$), dose and time dependently reduced blood glucose level, increased body weights of rats and had positive effect on organ weights and organ volume. The results in this study showed that *Citrullus lanatus* seeds are rich in zinc, selenium, manganese, iron, alkaloids, flavonoids, saponins, cardiac glycosides, steroids/triterpenoids, and tannins. They may therefore serve as good sources of these trace mineral nutrients and bio actives for nutritional and medicinal purposes relating to diabetes management.

Key words: Trace mineral, phytochemical, antidiabetic effect, alloxan, *Citrullus lanatus* seeds,

1. INTRODUCTION

New products based on resources of biological origin could be useful in making of food supplements, drugs and animal feeds (Okoroh *et al.*, 2021). Recently, the scientific study on the nutritional and nutraceutical values of naturally processed tropical seeds is very important because people will be encouraged via scientific information from the research to consume greater quantity of foods rich in seeds and nuts prepared in different forms which will eventually provide them with a better balance of nutrients to enhance health status (Okoroh *et al.*, 2021). According to Okoroh *et al.*, (2021), diabetes mellitus is a non-communicable disease which has been singled out as a major factor in the endocrine region of the biosystem responsible for the crisis in the metabolism of biomolecules such as fats, carbohydrates and proteins. Diabetes mellitus is a contributory factor to

impaired vision, stroke, kidney failure, cardiovascular diseases (WHO, 2016), Its prevalence has been rapidly on the increase particularly among middle- and low- income nations like Nigeria and WHO(2016), highlighted diabetes to be the 7th leading cause of deaths by 2030. The implication is that diabetes mellitus presents a major challenge to researchers and health care systems around the globe. Diabetes mellitus is defined as a group of metabolic diseases of endocrine origin indicated when there is high glucose level in the blood over a prolonged period and large amount of sugar in urine detected because of complete or relative lack of insulin resulting from the impairment of insulin secretion, insulin action or both (WHO, 2014). Its symptoms include osmotic diuresis which eventually causes excessive loss of water from tissues, increased thirst, hunger, and high concentration of lipids in the blood (WHO, 2013). Today, Insulin is mostly used in the treatment of diabetics and this is supported using a lot of anti-diabetic compounds including sulfonylurea, biguanides, and thiazolidinediones. These medications are costly, and difficult to access by the poor. Synthetic drugs are expensive and have side effects (Lee *et al.*, 2012).

Citrullus lanatus belongs to the family Cucurbitaceae, (Edwards *et al.*, 2003). Its English name is watermelon. In the flowering plant family called *Cucurbitaceae*, watermelon is a vine that trails and scrambles. The plant grows in climates such as the tropical and temperate climates. It produces a large edible fruit (berry), having hard rind and lacking internal division. The flesh is sweet and juicy with color ranging from deep red to pink. It has numerous black seeds. The fruit of water melon is usually eaten raw. It can also be pickled. Its rind can be washed and consumed fresh or cooked. The flesh is also consumed as juice or as part of beverages (Wehner *et al.*, 2001). Watermelon is grown in sandy loam soil rich in organic matter with good drainage and pH range of 6.5-7.5 (Kumar *et al.*, 2013). It is rich in essential micronutrients, macro-elements, vitamins and photochemical. Due to the high level of lycopene and potassium in watermelon, it can prevent stroke and cardiovascular diseases (Le *et al.*, 2005). Lycopene can also block inflammatory processes and works as an antioxidant to free radicals (Edwards *et al.*, 2003). It is rich in vitamin B6, Manganese and ascorbic acid. Watermelon fruit is rich in vitamin A and these nutrients are good for immunity and vision. As a result of the high water content of the fruit, it aids digestion and rehydration. Citrulline found in water melon seeds and rind is used in nitric oxide system in humans and has antioxidant and vasodilatation roles (Rimando *et al.*, 2005). The black water melon seeds are quite healthy and edible. They are rich in iron, zinc, protein and fiber. The seed has high arginine content showing that it has a lot of medicinal benefits (El-Adaway and Taha, 2001). They are rich in protein and essential fatty acids and there are prospects for the use of the seeds in the improvement of infant nutrition (Maynard, 2001) it is rich in antioxidants which reduce oxidative stress (Khaki *et al.*, 2013). It is rich in photochemical such as flavonoids which has been reported to have positive effect on pancreatic Beta-cells in terms of proliferation and secretion of insulin (Mahesh and Menon, 2004). Today, nations of the world are developing interest on making oral blood sugar reducing agents using parts of medicinal plants such as the leaves, fruits, roots, seeds and flowers with claims that they are cheaper, safer, more effective and without side effects, particularly for developing nations around the world. Diabetes mellitus and complications linked to it are still ravaging the world particularly

developing nations where synthetic drugs are not affordable. There is increasing interest on the use of natural products as alternative to orthodox counterpart because they contain bioactives that have medicinal values as well as nutritional importance. The bioactive compounds are called phytochemicals and they have been implicated in traditional medicine as natural healing agents that could be used in making phytomedicinal products around the world which may serve as cheap alternative with minimal or no side effect, for the management of diabetes and its related complications. This scientific study was aimed at the determination of the trace mineral, qualitative phytochemical composition and antidiabetic effect of ethanol extract of *Citrullus lanatus* seeds on diabetic rat

2. MATERIALS AND METHOD

SAMPLE COLLECTION AND PROCESSING

The seeds studied were those of *C. lanatus*. The seeds were extracted from the fruits of *C. lanatus* purchased from local farmers at Port Harcourt, Rivers State in July, 2021. The seeds were selected from the pulp, washed with clean water, exposed and allowed to dry under the sun. The whole seed (unpeeled) were ground into paste and stored in labeled air tight containers for analysis on dry weight basis.

ANALYSIS OF SAMPLE

The trace minerals zinc, manganese, iron and selenium were determined by atomic absorption spectrophotometry as described by AOAC (2019). Alkaloids, flavonoids, saponins, cardiac glycosides, steroids/triterpenoids, tannins, carbohydrates and oilstannins were determined according to the method of AOAC (2019).

PREPARATION OF *C. lanatus* SEEDS ETHANOL EXTRACT

The dried seeds were pulverized with a manual grinder and weighed with an electronic balance to obtain a mass of 400g (ground dry weight sample) which was well packaged and labeled. Ethanol extraction was carried out at Biochemistry laboratory, Gregory University, Uturu. To every mass of 200g of the pulverized material, 1000ml of 70% V/V of ethanol were used for soaking and the bottles were shaken intermittently. After 48hrs, first filtration process was done using clean white cotton material already immersed into the ethanol. Second filtration was done using Whatman No.1 filter paper. The filtrate was concentrated using a rotary evaporator at a temperature of 55°C and the concentrate was subjected to evaporation using a water bath regulated at a temperature of 55°C until

a paste which weighed 10g was obtained as extract. The paste was stored in a refrigerator until further experimental use. The percentage yield was 5g (w/v). This was calculated as follows:

$$\text{Extract percentage yield (\%)} = \frac{\text{weight of extract}}{\text{weight of dry ground power}} \times \frac{100}{1}$$

ANIMAL HANDLING

PILOT STUDY

Thirty (30) albino rats weighing 150-180g were collected from the animal house of the Department of Biochemistry, Gregory University, Uturu, Abia State, Nigeria. The rats were acclimatized for 7 days. The pilot study was carried out to determine the dose of alloxan monohydrate to be used for the induction of diabetes in the experimental rats. Three albino rats after acclimatization were administered three different doses of alloxan monohydrate (100mg/kg, 120mg/kg and 150mg/kg b.w. respectively) via intraperitoneal (I.P.) route. The rats were monitored for 72hr of fasting, blood sugar was determined via tail vein to establish diabetes in the rats through monitoring their hyperglycemic status.

EXPERIMENTAL DESIGN

Studies were conducted in compliance with the applicable laws and regulations.

INDUCTION OF DIABETES

After acclimatization of the animals for a period of 7 days, the rats were randomly sorted into five groups of five animals each. The five (5) rats in the normal group and the five (5) rats in the normal control group were placed on normal diet of guinea growers mash. The fifteen rats (n=5 rats/group) in the other three groups were fasted overnight and induced diabetes using a single intraperitoneal injection of alloxan (120mg/kg b.w.). Alloxan (Sigma, USA) at a dose of 120mg/kg b.w. was prepared in fresh and cold normal saline solution and administered immediately to the animals. The animals were first weighed using an electronic scale (TH 500) and their base line fasting blood glucose level taken using Fine Test Auto-coding Premium Blood Glucose Monitoring System and Blood Glucose Strips via tail vein cut before they were injected with alloxan. The animals in normal and normal control group were injected normal saline alone. After 72hr of administration, the rats were again fasted and blood collected via tail cutting and their fasting blood glucose level were tested which confirmed hyperglycemia. Metformin HCl and the extracts were given (1ml per animal) once daily by intragastric gavage to the experimental groups undergoing treatment while the normal group was given water only (1ml per animal) once daily and the normal control group received water and extract treatment. Fasting blood glucose and body weight were checked 48 hours after induction and on day 5 and 10 after treatment with extract and metformin HCl. The extracts and metformin HCl (reference drug) were kept in plastic bottles with cap tightly

sealed before and after each use, stored in the refrigerator, protected from direct sunlight to prevent spoilage throughout the time of animal treatment.

Table 1: Experimental design for the antidiabetic screening.

S/N	ID	Treatment
1.	Normal	Water + Basal Diet
2.	Normal control	Water + Basal Diet + CLE (500mg/kg b.w)
3.	Diabetic control	Water+BasalDiet+Alloxanmonohydrate (120mg/kg b.w.)
4.	Diabetic + CLE treatment(CLE ₅₀₀)	Water + Basal diet + Alloxan(120mg/kg b.w.) + CLE (500mg/kgb.w)
5.	Diabetic + reference treatment metformin HCl treatment 50mg/kgbw(MET ₅₀)	Water + Basal diet + Alloxan monohydrate (120mg/kgb.w.)+ metforminHCl(50mg/kg b.w.)

DETERMINATION OF ORGAN WEIGHTS AND SIZES, AND ORGAN WEIGHT INDICES

The carcasses of the rats were dissected and their lungs, kidney, heart, liver and spleen were excised and weighed. The sizes of the organs were also determined by water displacement method using eureka can. The can was filled to the mark with water and the organs were each completely immersed in the water. The volume of water displaced was recorded as the volume (cm³) of the organ.

STATISTICAL ANALYSIS OF DATA

Data obtained was statistically analyzed by a one-way analysis of variance (ANOVA) using SPSS/PC + package. Differences between means were compared by Duncan's (21) Multiple Range Test. Significance was accepted at a p-value of less than 0.05 (p <0.05).

3. RESULTS AND DISCUSSIONS

The trace mineral compositions of the seeds of *C.lanatus* are shown in Table 2. The mineral concentrations showed that iron had the highest value (8.31mg/kg) followed by zinc (5.78mg/kg), then manganese (4.28mg/kg). Selenium concentration was appreciable (0.85mg/kg). The seeds were found to be rich in iron, zinc, manganese and selenium. Manganese acts as a catalyst and co-factor in

a lot of enzymatic processes (Ghani *et al.*, 2012). Zinc is an essential trace mineral for growth, development and immune cell function (Deshpande *et al.*, 2013). Iron is important in the production of hemoglobin and addition of the seeds of *C.lanatus* in diets will help to build blood of people suffering from anemia. Okoroh *et al.*, (2017) also reported in their study that *P.ostreatus* are rich in iron, zinc and manganese. The values of iron , zinc and manganese reported by Olaniyi *et al.* , (2018) for leaves of *C.cujete*, Okoroh *etal.*,(2019) for *F.capensis* , Okoroh and Onuoha (2019) for *A.occidentale*, Okoroh *et al.*, (2013) for *L. africanaw* was higher than those reported in this study.

Table2 Trace- mineral composition (mg/kg) of seeds of *Citrullus lanatus*

Analyte	Composition (mg/kg D.W.)
Zinc (Zn)	5.78 ± 0.000
Manganese (Mn)	4.28±0.006
Iron (Fe)	8.31± 0.006
Selenium (Se)	0.85 ± 0.005

Values are means ± standard deviations of triplicate determinations where %D.W.means percentage dry weight

QUALITATIVE PHYTOCHEMICAL COMPOSITION OF THE SEEDS OF *Citrulluslanatus*

The result of the qualitative phytochemical screening of theseeds of *C.lonatus* is shown in Table 3.The presence of phytochemicals such as alkaloids, flavonoids, tannins, cardiac glycosides, carbohydrates, fixed oils, triterpenoids/steroids, saponnins and fixed oil in *C.lonatus* seeds reported in this study suggests its use as medicinal seeds. Thisreport is in consonant with the report by Oyeyemiet *al.*, (2017) for medicinal properties of secondary plant metabolites. The report in this study issimilar to that of Santhi *et al.*,(2011) on phytochemical profiling. Flavonoids are known to provide protection against degenerative diseases such as diabetes, cancer and heart diseases (Pandey, 2007). They have antioxidativeproperties (Kumaret *al.*, 2013). Alkaloid such as piperine reduces blood sugar level (Qui *et al.*, 2014). Tannins protect the body against free radicals (Yeet *al.*, 1999).The seeds of *C.lonatus* are rich in bio actives and may be used as nutraceutical

Table3: Qualitative phytochemical composition of seeds of *C.lanatus*

S/N	TEST ALKALOIDS	OBSERVATION	INFERENCE
i.	Dragendorff's test	Orange-red precipitate	+
ii.	Hager's test	Yellow precipitate	+
FLAVONOIDS			
i.	Shinod test	Reddish color	+
TRITERPENOID/STEROIDS			
i.	Liebermann-Burchard test	Color formation from violet to blue	+
ii.	Salkowski test	Reddish-brown color was formed at the interface	+

CARDENOLIDE			
i.	Keller killani test	A brown ring at the interface was observed. Below the brown ring was a violet ring. A greenish ring developed in the acetic acid layer and had a gradual spread at the layer.	+
ii.	Kedde test	An instant violet color which faded little by little through reddish brown to brownish-yellow leaving a white crystalline precipitate.	+
CARBOHYDRATES			
i.	Molisch test	A violet ring at the junction of two liquids was observed	+
ii.	Fehling's test	Brick red precipitate in trace was observed.	+
SAPONNINS			
i.	Frothing test	No froth was observed	+
TANNINS			
i.	FeCl ₃ test	No green or bluish-green or blue-black color	+
	Fixed oils	Paper was slightly translucent	+

+ = present, - = absent, ND = not detected.

BLOOD GLUCOSE OF RATS AND BODY WEIGHT OF RATS.

The alloxan induced diabetic rats (Dgroup,D+CLE500 and D+MET50) exhibited a significant increase in fasting blood glucose compared to the non-alloxan-treated rats in N group and N+CLE500 group. After CLE500 and MET50 treatment, the changes in blood glucose levels in different experimental animals are shown in Table4.After Day 5 of CLE500 and MET50 treatment , the level of glucose in D group , D +CLE500, D+MET50 were higher than those of N group and N+ CLE500 group respectively. On day 10 of CLE-500 and MET-50 treatment, the blood glucose level in Dgroup was significantly high ($p<0.05$) compared to that of all the other groups. The blood glucose level of N+CLE500 was significantly lower than that of the normal (Ngroup). The Metformin treated reference group (D +MET50) had lower blood glucose level than the D+CLE500

group and the values were significantly ($p < 0.05$) different. There was a significant ($p < 0.05$) reduction in blood glucose level after day 10, metformin and CLE500 treatment of the diabetic rats. On day 10, the body weights of rats on N+CLE500 and D+CLE500 increased like that of the normal rat compared to the body weights of rats in diabetic group which decreased. There was also a decrease in the body weights of rats treated with metformin on the 10th day of extract treatment.

The results showed that ethanol extract of the seeds of *C. lanatus* at 500mg/kg b.w. and metformin HCl lowered blood glucose level and increased the body weight in normal rats as well as treated diabetic rats. This effect may be because the plasma insulin level was now increased in diabetic rats which may have influenced the stimulation of the beta cells in islets of Langerhans (Winarsiet *et al.*, 2014). Chinmayet *et al.*, (2015) also reported that methanol extract of seeds of water melon reversed elevated blood glucose levels of fasting blood glucose and enhanced increase in body weight of diabetic rats. In diabetes, body weight may reduce because the body cells could not use glucose properly as source of energy. Proteins are used instead, leading to a decrease in body protein content and reduction in body weight (Guyton and Hall 2011). It is possible that extract restored protein metabolism and that made the weight of the diabetic animals and treated normal animals to increase. It is also possible that extract could have enhanced insulin secretion by beta cells or there is an increased sensitivity of target tissues for insulin or it may be because glucose metabolism has been improved (Chinmay *et al.*, 2015). Today around the world, diabetes is increasing in alarming rate because of people eat more of high density calorie foods and live sedentary life style. Diabetes is linked to abnormal lipid metabolism and has been taken as one of the major causes of atherosclerosis heart related diseases. (Biddinger and Kahn, 2006). Currently, WHO recommended the importance to investigate and explore hypoglycemic agents of plant origin due to the fact that plants used in tradi-medicine have less side effect when compared to their orthodox counterparts (Winnarsiet *et al.*, 2014). Plants are considered a more potent healer from ancient times because they promote repair mechanism in a natural way (Gurib-Farkim, 2006). Various herbs, rind of fruits, seeds, stems, leaves, fruiting bodies of mushrooms and spices have been indicated for the diabetes management (Son *et al.*, 2003). Okoroh *et al.*, (2021) reported that ethanol extracts of the fruiting bodies of organically cultivated *P. ostreatus* restored body weights of diabetic rats and reversed hyperglycemia suggesting the antidiabetic properties of the natural resources and its inclusion in the management of diabetes mellitus and its associated complications.

Table 4: Effect of Ethanol extract of *Citrullus lanatus* seeds on blood glucose levels(mg/DL) of the rats

Treatment group	72hr after alloxan treatment	Day5 (CLE treatment)	Day10(CLE treatment)
N	91.33 ± 4.99	103.32 ± 5.56	96.67 ± 10.87

N+CLE- 500	107.67 ± 4.92	95.33 ± 6.85	83.67 ± 3.68
D	435.33 ± 30.41	390.33 ± 58.45	381.00 ± 60.40
D+CLE- 500	266.33 ± 163.03	148.33 ± 172.08	130.00 ± 12.96
D + MET	350.00 ± 181.78	274.67 ± 105.51	115.67 ± 27.82

Values are means ± SD for five rats in each group of triplicate determinations. Where N = normal group ; N+CLE₅₀₀ group = Normal group treated with 500mg/kg b.w. extract dose ; Dgroup = diabetic group, D + CLE₅₀₀ = diabetic group treated with 500mg/kg b.w. extract dose; D+Met₁₅₀ group = diabetic group treated with 150mg/kg b.w. metformin HCl.

Table 5: Effect of ethanol extract of *Citrullus lanatus* seeds on body weights (g) of rats

Treatment group	Body Weights of rats before alloxan treatment	Body weights of rats (on day 10) of extract treatment
N	194.30± 12.23	207.67± 21.76
N + CLE 500	153.30 ± 3.40	193.33 ± 17.33
D	161.6 ± 4.50	142.67 ± 3.30
D+ CLE 500	145.60 ± 5.19	165.00 ± 7.25
D + MET	168.00 ± 10.61	157.33 ± 34.67

Values are means ± SD for five rats in each group of triplicate determinations. Where N = normal group ; N+CLE500 group = Normal group treated with 500mg/kg bw. Extract dose; Dgroup = diabetic group, D + CLE500 = diabetic group treated with 500mg/kg b.w. extract dose; D+Met₁₅₀ group = diabetic group treated with 150mg/kg b.w. metformin HCl.

Effect of ethanol extract of *C.lanatus* seeds on the organ weights and organ volume of diabetic rats

The effect of ethanol extract of *C.lanatus* seeds on organ weights are highlighted in Tables 6 and 7. The results showed that the extract dose at 500mg/kgb.w caused an increase in the weights of heart, kidney, liver, spleen and lungs of the diabetic group compared to the N group, N+CLE500 group, D+CLE500 group and D + MET₅₀₀ group respectively. Treatment with the extract had lesser reductive effect on the organ weights compared to the effect of metformin HCL. The results showed that the extract dose at 500mg/kgb.w caused an increase in the volumes of heart, kidney, liver, spleen and lungs of the diabetic group compared to the N group, N+CLE500 group, D+CLE500 group and D + MET500 group respectively. Treatment with the extract had lesser reductive effect on the organ volumes compared to the effect of metformin HCL. The results showed that organ weights and organ volumes increased in diabetic rats compared to the normal group but ethanol extract of

the seeds of *C.lanatus* at 500mg/kg b.w. and metformin HCl lowered organ weights and organ volumes in the treated groups but metformin treatment had more pronounced effect than the extract. Ezeugwu and Onoagbe (2018) reported that diabetes increases the organ weights of animals and this report is in consonant with the result in this study. For liver, this could be due to NAFL while as a result of the negative effect of diabetes on the blood vessels linking the kidney, there could be infiltration. The organ then retains water and salt causing infiltration.

Table 6: Effect of ethanol extract of *C.lanatus* seeds on the organ weights of diabetic rats

Group	Heart	Kidney	Liver	Spleen	Lungs
N	0.67 ±0.003	1.02±0.06	7.69±0.29	0.52±0.02	1.44±0.05
N + CLE500	0.67±0.003	1.12±0.23	7.00±1.40	0.55±0.08	1.59±0.29
D	0.81±0.81	1.49±0.19	8.46±1.34	1.35±0.42	2.14±0.41
D+CLE500	0.80±0.12	1.44±0.22	7.93± 1.76	1.19±0.44	1.92±0.34
D+MET50	0.82±0.21	1.09±0.19	6.01±0.43	0.60±0.03	1.30±0.32

Values are means ± SD for five rats in each group of triplicate determinations. Where N = normal group; N+CLE₅₀₀ group = Normal group treated with 500mg/kg bw extract dose; D group = diabetic group, D + CLE500 = diabetic group treated with 500mg/kg b.w. extract dose; D+Met₁₅₀ group = diabetic group treated with 150mg/kg b.w. metformin HCl.

Table7: Effects of Ethanol extract of *C.lanatus* seeds on the organ volume of diabetic rats

Group	Heart	Kidney	Liver	Spleen	Lungs
N	0.63±0.02	0.90±0.05	7.00±0.41	0.52±0.02	1.47±0.05
N + CLE500	0.67±0.04	1.03±0.19	6.93±1.56	0.57±0.55	1.37±0.35
D	0.91±0.27	1.70±0.28	8.60±1.33	1.63±0.59	2.80±0.22
D+CLE500	0.64±0.05	1.63±0.26	8.20±1.84	1.65±0.58	1.83±0.62
D+MET50	0.67±0.17	1.10±0.28	6.00±0.43	0.58±0.09	1.00±0.41

Values are means ± SD for five rats in each group of triplicate determinations. Where N = normal group; N+CLE500 group = Normal group treated with 500mg/kg b.w. extract dose ; D group = diabetic group, D + CLE500 = diabetic group treated with 500mg/kg b.w. extract dose; D+Met 150 group = diabetic group treated with 150mg/kg b.w. metformin HCl.

CONCLUSION

The study showed that the seeds of *C.lanatus* are rich in trace minerals such as zinc, iron, selenium and manganese and bioactive compounds such as alkaloids, flavonoids, tannins, cardiac glycosides, carbohydrates, fixed oils, triterpenoids/ steroids, saponnins and fixed oil .The results also showed that *C.lanatus* seeds possess antihyperglycemic activities in alloxan induced diabetic rats

These results therefore suggest that the seeds of *C.lanatus* are good sources of these trace mineral nutrients and bioactive compounds. The consumption of *C.lanatus* seeds may help to meet the nutritional and medicinal needs particularly in diabetics.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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