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Effects of Ethanol Extracts of *S. aethiopicum* Stalks on Lipid Profile and Haematological Parameters of Wistar Albino Rats

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Ogunka-Nnoka, C. U. *Onyegeme-OkerentaB. M., Omeje, H. C.

Department of Biochemistry, University of Port Harcourt, P.M.B. 5323, Port Harcourt, Nigeria

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ABSTRACT

The effects of ethanol extract of *Solanum aethiopicun* stalks on hematological parameters and lipid profile in albino rats were evaluated. Twenty (20) albino rats were randomly distributed into four major treatment groups each containing five rats. The study lasted for 21days. Rats in groups B, C, and D were fed extract of *S. aethiopicum* in the concentrations of 200, 500 and 1000 mg/kg respectively; after acute toxicity, the result proved that the extract is non-toxic even at LD_{50} greater than 5000 mg/kg. Group A was fed with commercial rat feed. The extracts resulted in a significant ($p<0.05$) reduction in plasma cholesterol, triacylglycerol, LDL cholesterol, and VLDL cholesterol. A significant ($p<0.05$) increase in HDL cholesterol was also observed. Hematological indices were determined using an automated haemacytometer machine. The results obtained for hematology, revealed significance effect ($p<0.05$) on PCV, RBC, and Hb. WBC showed non-significant ($p>0.05$) increase. This work demonstrated the ability of ethanol extract of *S. aethiopicum* stalks to improve the lipid profile of the plasma thereby lowering the risk of cardiovascular diseases.

INTRODUCTION

Solanum aethiopicum, commonly called African eggplant or garden egg, is among the oldest vegetables indigenous to tropical Africa. It is widely cultivated across West Africa especially for its nutritional, medicinal and economic values of the leaves and fruits. The fruit may be consumed freshly raw, dried, cooked and in salad form. It is one of the most important vegetable crops in West Africa as it is consumed daily and remains a source of income for many rural dwellers (Nwodo *et al.*, 2013).). Although the plant is seasonal, it is grown in all parts of Nigeria. The existing ethnic groups in Nigeria describe it with different names, for instance, the Igbos of South-eastern Nigeria call the fruit "Aṅara" "Afufa" or "Mkpuruofe", the Yorubas of South-western Nigeria call it "Igbagba" while the Hausas of Northern Nigeria call it "Dauta".

Garden egg (*Solanum aethiopicum)* also known as Ethiopian eggplant or scarlet eggplant is a vegetable crop belonging to the family *solanaceae.* The genius Solanum includes both the edible and non-edible species. The family is one of the largest and most important families of vegetable grown for their fruits (Prophens *et al.*, 2005). They are native to sub-Sahara Africa and are essentially tropical in origin. *S. aethiopicum* is of high edible quality. The fruits can be eaten fresh without cooking and have a long history of consumption in West Africa (Bonsu *et al.*, 2002). Depending on the type, either the leaves and the young shoots or the fruits or both are eaten. Fruits and vegetables are important to the body (Olusanya, 2008).

Aside from their nutritional roles in complimenting staple foods to form balanced diets, they also influence biochemical parameters in the body (Sofawara 1993). Such influence when positive helps the body to fight many disease conditions (Elujoba *et al*., 2005). Report has shown that *S. aethiopicum* possesses ulcer protecting properties against experimentally induced ulcers in rats (Ezeugwu *et al.*, 2004*),* has a reducing effect on lipid profile of Wister albino induced lipidemia (Nwodo *et al.*, 2013*),* and showed weight reducing effect as well as hypolipidemic properties (Ossamul *et al.*, 2014*).* They are used to treat severe pain resulting in periodic spasm in an abdominal organ and blood pressure (Grubben 2004). Other reports on the pharmacological activity of the plant show that it has purgative (Saba *et al* 2003), sedative and anti-diabetic effects (Ezeugwu *et al.*, 2004*).* The leafy part of *Solanum aethiopicum* is also applied to areas of skin disease, infections, and sores (Oliver-Beaver, 1989). Considering the rate at which the fruit of this plant is being consumed within Nigeria,

there is the need to look at the effect of the fruit stalk, which is a waste on some biochemical parameters.

In indigenous medicine, *S. aethiopicum* has a wide range of utilization from weight reduction to treatment of several ailments including asthma, allergic disease, swollen joint pains, gastro-esophageal reflux disease, constipation, and dyspepsia. Scientific studies have supported the traditional use of this plant in treating inflammation, asthma, glaucoma, diabetes and excessive weight gain (Anosike *et al.,* 2012*).* The fruit is easily eaten as a snack and it has been reported to be high in phytochemicals like saponins, flavonoids, tannins and ascorbic acid (Nwodo *et al.,* 2013*).*

Studies have shown that dyslipidemia-associated non-communicable diseases like diabetes and obesity are on the increase in the developing world and a continuous study is required to identify indigenous plant materials that can mitigate against, or at least useful in the management of, dyslipidemia (Dalal 2011, Nwodo *et al.,* 2013).

In developing countries, remedies from plants are readily used in the treatment of various kinds of diseases. Different medicinal plants possess diverse therapeutic potential as no single plant has all the medicinal properties (Ghasi *et al.,* 2011). Many of the medicinal potentials of plants used in folkloric medicine have been subjected to scientific investigation and this has warranted their widespread use as an alternative or complement to orthodox medicines. However, the medicinal potential of African flora is yet to be fully explored. Some plants of the African vegetation are still been discovered for their medicinal properties. This study was aimed at determining the qualitative and quantitative phytochemical composition of ethanol extract of *Solanum aethiopicum* stalk as well as evaluating its effect on the lipid profile and hematology indices of Wistar albino rats.

MATERIALS AND METHODS

Collection and Identification of Specimens

Fruits of *S. aethiopicum,* used for this study, were purchased from Ogige market, Nsukka, and identified by Mr. Ezugwu Paul, of the Centre for Development and Bioresearch Institute (CDBI), Nsukka.

Preparation of Ethanol Extract of *S.aethiopicum*.

The ethanol extract was prepared using the wet method of extraction. One kilogram of the dry stalks of the plant was blended in 1.5 litres of ethanol (96%) with an electric blender and transferred into the amber colored bottle and kept in a cool (4^0C) dark compartment for 72 hours. The mixture was filtered using a cheese material and thereafter with Whatman No 1filter paper. The extract was concentrated using a rotary evaporator at 37-40^{o}C and dried completely in a desiccator containing a self-indicating silica.

Phytochemical screening

Phytochemical screenings were carried out on the powdered ethanol stalk extract using standard procedures to identify the constituents as described by Sofowara, 1993; Trease and Evans, 2002).

Test for Alkaloids:

About 0.5 g of crude powder was defatted with 5% ethyl ether for 15 minutes. The defatted sample was extracted for 20 min with 5 ml of aqueous HCl on a boiling water bath. The resulting mixture was centrifuged for 10 minutes at 3000 rpm. One milliliter (1 ml) of the filtrate was treated with few drops of Mayer's reagent and another 1 ml with Dragendroff's reagent and turbidity was observed.

Test for Tannins

About 0.5 g of the dried powdered samples were boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

Test for Terpenoids (Salkowski test)

Five milliliters (5 ml) of the extract was mixed in 2 ml of chloroform, and 3 ml concentrated H_2SO_4 was carefully added to form a layer. A reddish-brown coloration of the interface was formed to show positive results for the presence of terpenoids.

Test for Glycosides (Keller-Killani test)

Five milliliters (5 ml) of the extracts were treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with1 ml of concentrated sulphuric

acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout the thin layer.

Test for Steroids

Two ml of acetic anhydride was added to 0.5 g ethanolic extract of the sample with 2ml H_2SO_4. The color changed from violet to blue or green in some samples indicating the presence of steroids.

Test for Saponins

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of an emulsion.

Test for Flavonoids

Five milliliters (5 ml) of dilute ammonia solution was added to a portion of the aqueous filtrate of the plant extract followed by addition of concentrated H_2SO_4. A yellow coloration observed in each extract indicated the presence of flavonoids. The yellow coloration disappeared on standing. Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow coloration was observed indicating the presence of flavonoids. A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration is positive for flavonoids.

Extraction of phytochemicals

The plant sample (1g) was transferred into a test tube and ethanol (15ml) and 50% m/v potassium hydroxide (10ml) was added. The test tube was left to stand in a water bath maintained at 60℃ for 60 minutes. The content of the test tube was emptied into a separatory funnel after this time. The reaction product was washed successively with 20ml of ethanol, 10ml of cold water, 10ml of hot water and 3ml of hexane. The extract was finally washed three times with 10ml of 10% v/v ethanol aqueous solution. The solution was dried with anhydrous sodium sulfate and solubilized with 100ul of pyridine. Afterward, it was transferred to a vial for analysis.

Quantification by Gas Chromatography

The analysis was carried out using a BUCK M910 Gas chromatography equipped with a flame ionization detector (FID). A RESTEK 15 meter MXT-1 column was used. The injector temperature was 280˚c and 2ul of the sample was injected at a linear velocity of $30cms^{-1}$. Helium, 5.0 Pas was the carrier gas maintained at a flow rate of $40mlmin^{-1}$. The initial temperature of the oven was 200˚c and was increased to 330c at a rate at 3˚c min^{-1} and was kept at this temperature for 15min. The detector operated at a temperature of 320˚c. The ratio of the area and mass of internal standard to area and mass of identified phytochemical was the means by which the phytochemicals were determined. The concentration was expressed in µg/g.

Animals

Wistar Albino rats (110-130g) were procured from a private farm (Ijeoma Rodent Farms) in Aluu, Rivers state and transported to the research site. They were housed in plastic boxes and acclimatized for 9days in a controlled environment (temperature 25±2 ^{o}C), with standard laboratory diet and water *adlibidum*

Experimental Design

Twenty adult Wistar rats were randomly divided into four (4) groups; one (1) control and three (3) treatment groups. The rats in group A, received only food and water, while the treatment groups B, C and D were administered with graded doses of the *S. aethiopicum* stalk extract of 200, 500 and 1000 mg/kg body weight respectively. The animals were fed orally once daily for 21days using rat oral cannula. This method was similar to the method of Oyeyemi, *et al.*, (2008).

Acute Toxicity Studies

The acute toxicity studies (LD_{50}) of *Solanum aethiopicum* stalks was determined using the method of Lorke, 1983. The studies were done in two phases. In the first phase, 9 rats were used. The rats were randomly divided into three groups having 3 rats in each group. Group 1 received 200mg/Kg, group 2received 500mg/Kg and group 3 received 1000mg/Kg via oral route respectively, and observed for signs of toxicity and death for 24 hours. In the second phase, 4 rats were used and consist of 4 groups with a rat in each group. Group 1 received 1500mg/Kg, group 2 received 3000mg/Kg, group 3 received 4500mg/Kg and group 4

received 5000mg/Kg. The median lethal dose (LD_{50}) was determined at the end of the second phase.

Blood Sample Collection

At the end of the 21days after an overnight fast, the animals were sacrificed anaesthetically by exposure to chloroform vapor for three minutes. Blood samples for each animal were taken in lithium heparin and EDTA bottles for investigation of lipid and hematological parameters. Hematology parameters investigated include: Packed Cell Volume (PCV), Red Blood Cell (RBC) count and white blood cell (WBC) and erythrocyte sedimentation ratio (ESR) while that of lipids include: Total Cholesterol, Triglycerides, LDL and HDL.

Determination of hematological parameters

The determination of hematological parameters was carried out according to the method described by Osim *et al.,* (2004) and that of Lewis *et al*., (2001).

Determination of Lipid Profile

Total Cholesterol, Triglycerides, LDL and HDL were analyzed by kinetic methods kits from Randox, (United Kingdom) using a double-beam spectrophotometer.

Statistical Analysis

The statistical package of social sciences (SPSS) software version 18.0 (SPSS) was used. The results were evaluated using analysis of variance (ANOVA) and were presented as the mean value ± SEM (standard error of the mean) for the control and experimental rats. Differences among the means for the groups were assessed using the Duncan's Multiple Range Test to determine which mean values were significantly different at $p<0.05$.

RESULTS

Phytochemical Composition

The preliminary phytochemical screening of the ethanolic extract of *Solanum aethiopicum* stalks conducted indicated the presence of alkaloids, flavonoids, steroids, tannins, and saponins. Table.1 shows the result of the qualitative and quantitative phytochemical composition of ethanol extract of *Solanum aehiopicum* stalks. It also showed that the alkaloids subclass of secondary metabolites were the most abundant in the plant.

Table 1: Qualitative and Quantitative Phytochemical Composition of Ethanol Extract of *Solanum aehiopicum* stalks

Phytochemicals	Qualitative Composition	Quantitative Concentration (%)
Alkaloids	+++	6.43
Flavonoids	++	0.83
Tannins	+	0.27
Terpenoids/Steroids	++	2.0
Saponins	+++	4.38

Key: + = present; - = absent

Acute Toxicity Result

During the experimental procedure, no deaths, no locomotor activity alteration, or any other clinical signs of toxicity were observed in any of the groups in both phases even at a dose of 5000mg/kg. The administration of different doses of *S. aethiopicum* stalk extract up to 5000mg/kg body weight did not produce any significant changes in responses linked to either behavioral or neurology. This proved the non-toxic nature of the *S. aethiopicum* stalk extract as all the treated rats were observed to be normal and there was no case of mortality or toxicity reaction at any of the doses until the end of the study. Therefore, LD_{50}>5000mg/Kg, indicate that the extract is safe and non-toxic.

Lipid profile

The results of the effect of the plant stalk extract on lipid profile are presented in Table 2.

Table 2: Result of ethanol extract of *S.aethiopicum* on lipid parameters of rat (mg/dl)

GROUP	TG	T. CHOL	HDL	LDL	VLDL
GroupA (Control)	232.00±15.12^{bcd}	137.80±4.85^{cd}	46.40±5.27	64.84±4.68^{bcd}	47.00±3.04^{bcd}
Group B (200mg/kg)	395.20±13.96^{a}	130.00±2.30	55.20±1.28	27.80±0.83^{acd}	79.04±2.79^{a}
Group C (500mg/kg)	395.00±16.52^{a}	120.60±1.86^{a}	56.40±3.43	16.48±1.54^{ab}	79.00±3.30^{a}
Group D 1000mg/kg)	424.00±28.10^{a}	117.80±2.65^{a}	60.00±3.18	9.80±0.96^{ab}	84.80±5.62^{a}

Values are mean±SEM, n=5.Values with different superscript in the row differ significantly ($p<0.05$)

The effect of different concentrations of the plant stalk extract on triglyceride level, ranged from 232.00- 424.00mg/dl. The treated groups, did not show any significant ($p>0.05$) difference; but there was significant difference (p,0.05) when compared to the control. Total cholesterol showed that there was significant decrease ($p<0.05$) in the extract treated groups (B, C, and D) when compared to the control (group A). HDL for the control was 46.40±5.27. This was significantly increased ($p>0.05$) in the extract treated groups when compared to the control group. The LDL for the control group was 64.84±4.68. The LDL of the extract treated groups were significantly decreased ($p<0.05$) when compared with the control group. Results obtained for VLDL for the control group was 47.00±3.04. When the VLDL of the control group was compared to the treated groups, it was observed that there was a significant increase ($p<0.05$) in the extract treated groups. However, there was no significant difference between the treatment groups (B, C, and D).

Hematological parameters

The results of the effect of the plant stalk extract on some hematological indices are presented in Table 3.

Table 3: Result of ethanol extract of S. aethiopicum stalks on hematology.

GROUP	PCV (%)	RBC (10^6/ml)	WBC (10^9/ml)	HB (g/dl)	ESR (mm)	PLATELET (10^6/ml)
GroupA (Control)	37.60 ±0.68^{cd}	4.80 ±0.37^{bcd}	4.80 ±0.37^{a}	13.70 ±0.04^{cd}	2.20 ±0.37^{a}	9.20 ±0.58^{d}
Group B (200mg/kg)	42.20 ±0.02^{cd}	7.60 ±0.40^{acd}	5.00 ±0.32^{b}	0.96 ±0.11^{d}	2.00 ±0.31^{a}	10.40 ±0.74^{c}
Group C (500mg/kg)	48.60 ±0.93^{abd}	10.80 ±0.49^{abd}	5.20 ±0.58^{b}	14.44 ±0.06^{ad}	2.80 ±0.73^{a}	11.40 ±0.87^{b}
Group D (1000mg/kg)	55.20 ±1.85^{abc}	14.40 ±0.51^{abc}	5.60 ±0.24^{b}	16.06 ±0.30^{abc}	3.80 ±0.97^{b}	12.80 ±1.16^{a}

Values are mean±SEM, n=5. Values with different superscript in the row differ significantly ($p<0.05$)

The effect of different concentrations of the plant stalk extract on Hb and PCV showed a significant increase ($p<0.05$) for the treated groups (B, C, and D) when compared to the control. Results obtained showed that the RBC for the control varied significantly ($p>0.05$) with the treated groups; B, C, and D. The table also shows that the increase in RBC count was proportional to the concentration of the dose administered.

There was no statistically significant difference in the ESR of the treated groups when compared with the ESR of the control group. Similarly, there were no statistically significant differences in the white blood cell and platelet count of the extract treated groups when compared with the control group,

DISCUSSION

One of the risk factors that contribute to and predispose people to cardiovascular diseases is Hyperlipidemia (Chen *et al*; 2007). Ossamulu (2014) posits that excessive levels of total cholesterol and low-density lipoprotein cholesterol (LDL-C) promote atherosclerosis and other heart-related disorders. It is therefore important that approaches to lower these lipid concentrations are employed. The significant increase in the weight gain by rats in the experimental groups correlates with the findings of Anosike *et al.*, (2012) and Odetola *et al*., (2004) who reported that the increase in weight gain as of the animals indicates that the

Solanum aethiopicum are efficient in weight management. According to the observation of Edijala *et al*., (2005), garden eggplants, significantly reduced weight gain in rats fed on eggplant fruit compared to those that had oat and apple in both the mid-term and full-term studies. It may, therefore, be reported that appetite was not a factor that influenced the observed effects. The effect of eggplants on weight reduction may be due to their low energy density, which may be attributed to the high moisture, fiber and low-fat contents they possess (Roberts *et al*., 2006). This agrees with previous findings in the study of Ossamulu *et al*., (2014), who reported high moisture, fiber, and low fat contents for all the eggplant varieties evaluated.

This present study investigated the improvement of plasma lipid profile as well as the hematological parameters of Wistar albino rats by *S. aethiopicum* stalk extracts. Dyslipidaemia is known to be indicated by elevated plasma triacylglycerols, total cholesterol, LDL-cholesterol and VLDL-cholesterol with a concomitantly reduced level of HDL-cholesterol (Nwodo 2013). Dietary management with fruits has long been recommended as part of the scrupulous controls necessary to prevent and/or manage dyslipidemia; an abnormal level of body lipid (Mirmiran 2009). Hence, it is apparent from the results presented in this study and from results reported by other authors that fruits as if *S. aethiopicum* and its stalk inclusive have plasma lipid-lowering effects. Edijala *et al*. (2005) carried out an experiment with hypercholesterolemic rats fed *S.melongena (a* member *of* the Solanum family*)*, oat and apple and reported that *S. melongena* had the more hypolipidemic effect than apple and oat.

Nwodo *et al*., 2013, reported that there are two sources of cholesterol namely; the exogenous source from the diet and endogenous source where its synthesis is *de novo*. The efflux of cholesterol from these two sources into the plasma has been reported to be influenced by dietary factors. *S. aethiopicum* fruits have been reported to be rich in some of these dietary factors like saponins, alkaloids, flavonoids, and tannins as well as dietary fiber (Edijala *et al*., 2005), these are also contained in the stalks of the *S. aethiopicum*. Many studies have reported the ability of saponins to reduced plasma lipids (Whitehead *et al*., 2010). Elekofehintimi *et al*. (2012) reported that the hypolipidemic effect of saponin could be due to several mechanisms, which include a decrease in fatty acid, enhanced LDL receptors, activation of LCAT and lipase as well as of acetyl-CoA carboxylase.

Tannins like other polyphenolic compounds also possess a variety of other biological activities, such as reduction of plasma lipids, which might be due to the up-regulation of LDL receptor expression (Nwodo *et al* 2013), inhibition of hepatic lipid synthesis (Adeli *et al*., 2001) and lipoprotein secretion (Borradaile *et al*., 2003) an increase in cholesterol elimination via bile acids. These could have synergistically accounted for our observations as a decrease in LDL-cholesterol and triacylglycerols levels because of *S. aethiopicum* stalk administration. The decrease observed in the plasma total cholesterol level could also be due to the exogenous regulation.

Saponins are known to inhibit intestinal cholesterol absorption by binding to bile acids thereby leading to increased bile acid excretion (Nwodo *et al*., 2013). The consequence of this is elevated HDL-cholesterol because of reverse cholesterol transport. This is consistent with our findings, as the rats fed *S. aethiopicum* stalk extract had elevated HDL-cholesterol. It is also worth noting that saponins have been reported to lower the expression of HMG-CoA reductase at both mRNA and protein levels. Consequently, endogenous cholesterol synthesis is reduced and cholesterol is mobilized from extra-hepatic tissues to the liver for bile acid biosynthesis. This is often indicated by an increase in HDL-cholesterol.

The importance of hematology test in the assessment of blood relating functions of substances that enter the body cannot be overstated. Results of hematology as shown in Table 3, revealed that Hb levels increased apparently in the test groups and became significant ($p<0.05$) in test groups C and D when compared to the control. Increase in Hb level is normally followed by a corresponding increase in PCV level. This could be the cause of the observed significant ($p<0.05$) increase in PCV levels of test groups against the control in the present study. The increased Hb and PCV levels in this study could be that the studied stalk extract does not induce anemia. The WBC and its differentials are known to protect the body against the foreign body (Guyton 2000). Their increase in the system is considered as a defensive mechanism by the immune system (Sliverd 2007). The ESR is useful as a screening test for any acute or chronic infectious conditions with marked alteration in plasma protein concentration. Serial ESR can be used to monitor disease progression or treatment. Immunoglobulins are affected in raised ESR while increased plasma albumin slows the ESR (Duru 2009). The ESR levels in test groups were insignificantly affected ($p<0.05$) when compared to the control group. It could be that the studied stalk extract, did not affect the ESR of the test rats in the present.

CONCLUSION

The present study has shown the effect of *Solanum aethiopicum* stalk extract on hematology and lipid profile. The observation made so far in the present study revealed a non-negative effect on the hematology and lipid profile function parameters of therats used. The implication could be that humans that consume this fruit are exposed to the same effect. The results demonstrated that eggplants have great medicinal values such that they could be useful in health or pharmaceutical industries. Nwodo 2013, reported that soluble fibers could be fermented in the colon into short-chain fatty acids which in turn lower the synthesis of cholesterol and triacylglycerols. This could have also contributed to the reduced plasma total cholesterol, triacylglycerol, LDL-cholesterol and VLDL-cholesterol levels. The necessity of reduced levels of these lipids in managing dyslipidemia, especially in atherogenic condition is well known (Rotimi *et al*., 2012). Our results, therefore, suggest that *S. aethiopicum* stalks may be beneficial in the dietary management of dyslipidemia and weight.

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