

Solanum macrocarpon (African eggplant) and Abelmoschus esculentus (Okra) fruits prevent weight gain and improve lipid profile in high-fat diet-fed Wistar rats.

Mutiat B. Ibrahim^{1*}, Ridwan A. Lawal², Andrea O. Akinjo³, Ifreke M. Luke¹

¹Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Nigeria ²Department of Biochemistry, College of Medicine, University of Lagos, Nigeria ³Department of Anatomic and Molecular Pathology, Lagos University Teaching Hospital, Nigeria

ARTICLE INFO

Article histo	ry:				
Received	28 September 2023				
Revised	09 November 2023				
Accepted	29 December 2023				
Online	30 April 2024				
Published	*				
Keywords:					
Weight Red	uction,				
Lipid Metabolism,					
High-fat Diet,					
Solanum Macrocarpon,					
Abelmoschu	s Esculentus				
*Corresponding mibrahim@unila https://orcid.org/ +234803324691	ag.edu.ng /0000-0001-5900-7950				

ABSTRACT

Background: Natural products or functional supplements from edible plants have served as an attractive strategy for weight loss. *Solanum macrocarpon* L. (Solanaceae) and *Abelmoschus esculentus* (L.) Moench have been used in indigenous medicine for weight reduction and to treat several diseased conditions. This study evaluates the potential of *S. macrocarpon* and *A. esculentus* in weight reduction and lipid metabolism as well as revealing their phytochemical profile.

Methods: Thirty-six rats were randomly divided into six groups of six rats each. Group 1 rats were fed with a 100% standard rodent diet (StD) throughout the experimental period. Rats in groups 2 to 6 were fed with a high-fat diet (HF-StD) for 4 weeks to induce obesity. Following obesity induction, the HF-StD was removed and obese rats in group 2 were given StD; groups 3 and 4 rats were fed with StD supplemented with 10% and 20% *S. macrocarpon* (SM) and groups 5 and 6 rats were fed with StD supplemented with 10% and 20% *A. esculentus* (AE) for a period of 8 weeks. Food uptake by the rats and their body and liver weights were monitored. Blood samples of rats were collected for serum lipid profile analysis. Gas chromatography/mass spectroscopy (GC/MS) was employed for phytochemical profiling of the plant samples.

Results: The supplementation of diet with *S. macrocarpon* and *A. esculentus* affected food intake, prevented body and liver weight gain and lowered plasma total cholesterol (TCHO), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c) and triglycerides (TG) levels of the rats. Phenolic acid derivatives and long-chain fatty acids were detected in the plant samples.

Conclusion: *S. macrocarpon* and *A. esculentus* show potential for body weight reduction and regulation of lipid metabolism and warrant further research.

1. Introduction

The buildup of a large amount of fat in the human body has been linked to various health risks such as hypertension, osteoarthritis, diabetes mellitus, fatty liver disease, cancer, and obesity. The World Health Organization (WHO) characterizes obesity and overweight as abnormal or excessive fat accumulation that arises from an energy imbalance between calorie intake and calorie expenditure, leading to increased lipid concentration in the blood and enlarged fat mass ^{1,2}. Body mass index (BMI) defined as an individual's weight in kilograms divided by the square of the individual's height in meters (Kg/m²) is a simple index of weight for height commonly used to classify overweight and obesity in adults ³.

The estimates for global levels of overweight and obesity (BMI ≥ 25 kg/m2), referred to as high BMI suggest that over 4 billion people may be affected by 2035, compared to over 2.6 billion in 2020⁴. The prevalence of obesity

worldwide nearly tripled between 1975 and 2016. In 2016, more than 1.9 billion adults aged 18 years and older were overweight of which over 650 million adults were obese. In 2019, an estimated 38.2 million children under the age of 5 years were overweight or obese. Once considered a high-income country problem, overweight and obesity are on the increase in low- and middle-income countries, particularly in urban settings⁵.

The increase in the prevalence of obesity necessitates an increased need for therapeutic and preventive strategies. It is evident that the development of obesity is caused by a disorder of lipid metabolism and glucose homeostasis ^{6,7}. Thus, the regulation of these two factors could be an important approach to controlling obesity ^{8,9}.

Natural products or functional supplements from edible plants have served as an attractive strategy for weight loss. Most herbal products and their derivatives are often obtained from crude plant extracts, which comprise a complex mixture of different plant secondary metabolites including phenols commonly found in plant fruits and vegetables. Many epidemiological studies have reported that the consumption of plant food with high phenolic content is associated with reduced incidence of chronic pathologies including obesity ^{10,11}. Gas-chromatographymass spectrometry (GC-MS) method used for the phytochemical analysis of plant extracts can be an interesting tool for testing the amount of some active principles in herbs used in the pharmaceutical or food industry¹².

Solanum macrocarpon L. (Solanaceae) locally known by the Yoruba people of Southwest Nigeria as Igba is a tropical perennial plant that is closely related to the eggplant, Solanum melongeta L. (Solanaceae). Commonly known as African eggplant or gboma eggplant, S. macrocarpon originated from West Africa and is widely distributed in Central and East Africa¹³. The plant is cultivated for its edible fruit and leaves which are sold in local markets as a food of medicinal importance. In Nigeria, the fruit is used as a laxative, and as a means to treat cardiac diseases 14,15 . S. macrocarpon has been used in indigenous medicine for weight reduction and for the treatment of several diseases such as asthma, allergic rhinitis, nasal catarrh, rheumatic disease, and swollen joint pains among others ^{15,16}. Previous pharmacological research has reported the effect of S. macrocarpon fruit on weight gain, blood glucose and liver glycogen in Wistar rats¹⁷. However, there is no report on the weight reduction effect of this plant in high-fat-induced rats.

Abelmoschus esculentus (L.) Moench is a fruit popularly known as okra or lady's finger and belongs to the Malvaceae family. The plant is cultivated in tropical, subtropical, and warm temperate climates in various countries and serves as a potential functional food hence it is commonly used in cooking. It is used in traditional medicine in the treatment of irritation of the stomach, intestines and kidneys and to treat worm infestations, dysentery, inflammation and weight reduction ^{18,19}. The antioxidant, anti-inflammatory, immunomodulatory, anticancer, antidiabetic, neuropharmacological and lipid-lowering activities of *A. esculentus* have been reported ^{20,21}. In this study, the weight reduction and lipid metabolism potential of *S. macrocarpon* and *A. esculentus* fruits in high-fat diet-fed rats as well as phytochemical analysis of the plants are being evaluated.

2. Materials and Methods

2.1 Plant Collection and Preparation

Fresh fruit samples of Solanum macrocarpon L. and Abelmoschus esculentus were obtained from a local market in Mile 12, Lagos in February 2022. The plants were identified and authenticated by Dr George I. Nodza at the herbarium of the Department of Botany, Faculty of Science, the University of Lagos where voucher specimen numbers LUH 9108 and LUH 9109 were obtained for S. macrocarpon and A. esculentus respectively. The fruits were selected and washed thoroughly under running tap water to remove dirt and unwanted particles. The stalks of the fruit were removed and the fleshy fruits were cut into small pieces, and air dried for a day followed by drying in a hot air oven at 40°C. The dried plant samples were pulverised in a mechanical grinder and the powdered dried fruit samples of S. macrocarpon and A. esculentus were maintained at room temperature till further use for in vivo study. About 100 g of the powdered plant samples were macerated separately in about 250 mL of absolute ethanol for 72 h at room temperature. The extracts were filtered through Whatman filter paper and evaporated to dryness in a water bath at 40 °C. The dried ethanolic plant extracts were stored at room temperature until further use for phytochemical analysis.

2.2 Experimental diets

Four diets were used in this study. A standard rodent diet (Livestock Feeds PLC, Ibadan, Oyo State) for experimentation was considered the standard rodent diet (StD). Another diet was a high-fat diet (HF-StD) to induce obesity, which was prepared by frying StD (50%) with commercial pork lard (50%) for 20 mins. To avoid autooxidation of the fat components, the HF-StD was freshly prepared each day. The other two diets consisted of StD supplemented with different levels ($10\% \text{ "/}_w$ and $20\% \text{ "/}_w$) of dried *S. macrocarpon* and *A. esculentus* fruits samples and these diets were considered as treatment diets for obesity.

2.3 Experimental Animals

Male and female Wistar rats weighing 150 - 175 g were obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria. The animals were placed in cages and kept for a minimum of 7 days to allow for acclimatization to the laboratory conditions. The animal room was ventilated with 12-h periods of light and dark conditions at a temperature of $23^{\circ}C \pm 2^{\circ}C$ and animals were fed orally with StD and water ad libitum. The animal cage bedding and feeding water bottles were cleaned on a daily basis.

The research protocols used in this experiment were in accordance with the provisions of the United States National Academy of Sciences Guide for the Care and Use of Laboratory Animals²² and an approved protocol by the Health Research Ethics Committee of the College of Medicine, University of Lagos, Nigeria (CMUL/ACUREC/03/23/1149)

2.4 Experimental design

Group 1: Healthy control. Six rats were fed orally in groups with 100% StD and water ad libitum throughout the whole experiment (StD-control).

Group 2-6: Thirty rats were fed orally in groups of six rats per group with HF-StD for 4 weeks to induce obesity. The rats were weighed after 4 weeks of consumption of HF-StD to ascertain the induction of obesity. After 4 weeks of obesity induction, the HF-StD was removed and obese rats were fed orally with StD and StD-supplemented diets for 8 weeks.

Group 2: obese rats fed orally with 100% StD (Ob-StD-control)

Group 3: Obese rats fed orally with 90 g StD supplemented with 10 g dried fruit sample of *S. macrocarpon* (Ob-10% $^{\text{w}}/_{\text{w}}$ SM/StD)

Group 4: Obese rats fed orally with 80 g StD supplemented with 20 g dried fruit sample of *S. macrocarpon* (Ob-20% $^{\text{w}}/_{\text{w}}$ SM/StD)

Group 5: Obese rats fed orally with 90 g StD supplemented with 10 g dried fruit sample of *A. esculentus* (Ob-10% $^{\text{w}}/_{\text{w}}$ AE/StD)

Group 6: Obese rats fed orally with 80 g StD supplemented with 20 g dried fruit sample of *A. esculentus* (Ob-20% $^{\text{w}}/_{\text{w}}$ AE/StD)

The dietary intervention lasted 8 weeks with oral administration of the diets and water ad libitum. During the 8-week treatment period, the food intake of the rats was measured daily and their body weights were monitored every week. After 8 weeks of consumption of the experimental diets, all animals were fasted of feed but left with drinking water ad libitum for 12 h and were sacrificed by decapitation under inhaled diethyl ether anaesthesia to obtain blood and hepatic tissue ²³. Blood samples from rats were collected by retro-orbital puncture using capillary tubes into non-heparinised centrifuge tubes for the biochemical study. A deep longitudinal incision was made into the ventral surface of the abdomen and thorax of the sacrificed rats and by blunt dissection of the muscles and fasciae, the liver was exposed and harvested.

2.5 Measurement of food intake, body and liver weights

The daily consumption of diet by the rats for the 8-week treatment period was recorded by taking the difference between the amount of feed given and the amount of left-over feed. The rats were weighed individually weekly throughout the experimental period and the % weight change for each animal at the end of the study was calculated as % Weight change = (Difference between interval body weight and initial body weight \div initial body weight) × 100. The harvested liver weight was standardized for 100 g body weight of individual rats.

2.6 Measurement of serum biochemical parameters

The serum biochemical profiles including total cholesterol (TCHO), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), triglyceride (TG), total bilirubin (TBIL) total protein (TP), and the enzyme activity of albumin (ALB) as well as the concentration of electrolytes blood urea nitrogen (BUN) and creatinine (Cr), were determined in the serum samples of rats using enzymatic kits (Randox Laboratories Limited, Crumlin, County Antrim, BT29 4QY, UK) performed according to manufacturer's instruction and on XL-640 fully automatic clinical chemistry analyzer (ERBA[®] Mannheim, Czech Republic).

2.7 Histological analysis of liver tissue

The harvested liver tissues were weighed and fixed in a 10% buffered formalin solution to observe for possible histopathology changes. After fixation, the tissues were exposed to routine processing, embedded in paraffin and sectioned at about 3 μ m²⁴. The hepatic morphology was assessed by a pathologist from the tissue sections stained with hematoxylin and eosin stain using Leica DM 500 microscope attached to a camera Leica ICC50 HD (Leica Microsystems Ltd., Switzerland) and photographed at a final magnification of 100x.

2.8 Identification of bioactive constituents by GC/MS

The phytochemical evaluation of the ethanolic plant extracts was performed on Gas Chromatography/ Mass spectrometry (GC/MS) equipment using Agilent 7820A gas chromatograph coupled to an Agilent 5975C inert mass spectrometer with triple axis detector operated in an electron impact mode with ionization energy of 70 eV. The separation of compounds was on the stationary phase HP-5 capillary column coated with 5% phenyl methyl siloxane (30 m length x 0.32 mm diameter x 0.25 µm film thickness). Pure helium gas was used as the carrier gas at a constant flow rate of 1.487 mL/min with an initial nominal pressure of 1.490 psi and an average velocity of 44.22 cm/sec. 1 µL of the samples were injected in splitless mode at an injection temperature of 300 °C. The column oven temperature started at 40 °C for 1 min then raised to 300 °C at the rate of 12 °C/min and held for 10 min. The total elution time was 32.67 min with a 5 min solvent delay. Identification of phytochemicals present in the test samples was based on comparisons of their relative retention time (min) and mass spectra with a spectral database of known compounds obtained from the National Institute of Standards and Technology (NIST) library²⁵.

2.9 Statistical analysis

Data are expressed as the mean of six replicates \pm standard error of mean (SEM). Statistical significance at values of p < 0.05 among the experimental groups was analyzed with IBM SPSS Statistic version 29.0.1.0 (171) software using a one-way analysis of variance (ANOVA) followed by Tukey post hoc multiple comparisons.

3. Results

3.1 Effect of the plant-supplemented feed on the rats' body and organ weights and food intake

Tables 1 and 2 show the body weights of the experimental

animals pre- and post-treatment with standard feed (StD) supplemented with dried fruit samples of S. macrocarpon and A. esculentus. Prior to supplementation of feed with plant samples, a significant (p < 0.05, 0.01, 0.001) increase in average body weights (201.0 - 217.33 g) of rats in the HF-StD-fed groups was observed as compared to the rats in the StD-fed group (162.83 g). There was a percentage increase in body weights (21.59 % - 32.52%) of rats in the HF-StD-fed group with an increased total food intake by the rats (Figure 1). As shown in table 2, after the 8 weeks of treatment with standard feed (StD) supplemented with dried fruit samples of S. macrocarpon and A. esculentus, rats' body weight gain decreased (-19.19%) considerably in obese rats given StD supplemented with $10\%''_{w}$ of S. macrocarpon (Ob-10% "/ SM/ StD) compared to Ob-StDcontrol (obese rats given StD) group (-20.93%). Other treatment groups also showed a decrease in rats' body weight (-22.85% to -25.43%). A significant (p < 0.05, 0.01) decrease in total food intake by the rats was observed in obese rats given StD supplemented with 20% $^{\text{w}}/_{\text{w}}$ of S. macrocarpon (Ob-20% SM/ StD) and $20\%''_{w}A$. esculentus (Ob-20% AE/ StD) during the post-treatment period compared to Ob-StD group (Figure 2).

Table 3 showed an increased weight of the liver of rats in the Ob-StD-control group $(3.10 \pm 0.48 \text{ g})$ as compared to the StD-control group $(2.76 \pm 0.14 \text{ g})$. The liver weights of rats in the treatment groups were significantly (p < 0.05, 0.01) lowered with a range of 1.86 ± 0.11 to 2.58 ± 0.13 g as compared to the Ob-StD-control and StD-control groups.

3.2 Effect of the plant-supplemented feed on rat's serum biochemical parameters

A significant increase (p < 0.05, 0.01, 0.001) in the levels of TCHO, LDL-c, and TG and a non-significant (p > 0.05) increase in the levels of HDL-c and TBIL was observed in the treatment groups compared to the Ob-StD-control group and the StD-control groups (Table 4).

After treatment by supplementation of StD with *S.* macrocarpon and *A. esculentus*, the TCHO, LDL-c, HDL-c and TG levels were significantly (p < 0.05, 0.01, 0.001) decreased in the treatment groups (Table 4). The obese rats given StD supplemented with 20%^w/w of *S. macrocarpon* and 10% *A. esculentus* exhibited significant (p < 0.05, 0.001) lowered levels of TCHO (21.84 ± 2.55 and 25.17 ± 2.12 mg/dL) as compared to the StD-control and Ob-StDcontrol groups with TCHO levels of 36.93 ± 4.14 and 37.83 ± 2.90 mg/dL respectively. The Ob-20% ^w/_w SM/StD, Ob-

10% ^w/_w AE/StD and Ob-20% ^w/_w AE/ StD groups showed a significant (p < 0.01, 0.001) reduction of LDL-c levels to $4.44\pm0.53, 7.65\pm0.81$ and 8.40 ± 2.04 mg/dL as compared to 17.01 ± 2.12 and 18.37 ± 1.73 mg/dL observed in the StD-control and Ob-StD-control groups respectively. Treatment of obese rats given 20% S. macrocarpon, 10% A. esculentus and 20% A. esculentus supplemented diets significantly (p < 0.05, 0.01) lowered TG levels to 7.35 \pm $1.35, 5.34 \pm 0.99$ and 7.38 ± 1.64 g/dL compared to 12.51 ± 1.82 and 15.66 ± 2.95 mg/dL in the StD-control and Ob-StD-control groups respectively. Except for the Ob-10%^w/_w SM/StD group which showed a significant (p < 0.01) increase ($0.82 \pm 0.03 \text{ mg/dL}$), all the treatment groups showed a significant (p < 0.01, 0.001) decreased in TBIL levels to 0.10 ± 0.01 , 0.07 ± 0.02 and 0.10 ± 0.02 compared to 0.61 ± 0.05 and 0.75 ± 0.05 mg/dL in the StD-control and Ob-StD-control groups respectively. The levels of serum TP and ALB were non-significantly (p > 0.05) lowered in all the treatment groups except for the Ob-20% "/, SM/StD

group which showed a significant (p < 0.05) decrease in TP level to 6.41 ± 0.24 g/L compared to 7.26 ± 0.31 and 7.58 ± 0.16 g/L in the StD-control and Ob-StD-control groups respectively.

3.3 Effect of the plant-supplemented feed on histopathological assessment of rat's liver

Mild/moderate periportal inflammation within the hepatic lobule was observed in the liver tissue of rats in the Ob-10% $^{w}/_{w}$ AE/StD group. Rats in the Ob-10% $^{w}/_{w}$ SM/StD and Ob-20% $^{w}/_{w}$ AE/StD treatment groups also showed mild periportal lymphocytic infiltrate (Figure 3). There was no histopathological changes observed in the StD-control, Ob-StD-control and Ob-20% $^{w}/_{w}$ SM/StD groups (Table 3).

Table 1: Effect of HFD-	-StD food	on body w	eights of rats
	010 1004	011 0 0 0 0 1	engines of reves

Obesity induction period: Body weight (g)							
Parameters	StD fed rats	HF-StD-fed rate	8				
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	
Initial weight	135.17 ± 1.14	164.0 ± 2.03	172.17 ± 1.10	169.67 ± 1.71	170.33 ± 1.99	162.0 ± 1.29	
Induced obesity (4	162.83 ± 2.73	217.33 ± 4.73	212.83 ± 2.62**	$201.0\pm3.27\texttt{*}$	211.17 ± 3.40 **	$205.0 \pm 2.63*$	
weeks)							
Per cent weight	17.45 ± 7.12	32.52 ±13.44	23.62 ± 6.35	21.59 ± 8.82	23.97 ± 12.83	26.54 ± 6.02	
changes (%)							

Weight values are expressed as mean \pm SEM.* p < 0.05; ** p < 0.01; significant body weights difference between the rats fed orally with HFD-StD and the rats orally fed with StD in control group 1 (One-way ANOVA followed by Tukey post hoc test)

Table 2: Effect of S. macrocarpon and A. esculentus dried fruits ingestion on body weights of rats

Drug treatment period: Body weight (g)								
Parameters	Group 1:	Group 2: Obese -	Group 3: Obese-	Group 4: Obese -	Group 5: Obese -	Group 6: Obese -		
	StD - Control	StD - Control	$10\%^{\rm w}/_{\rm w}$ SM/ StD	$20\%^{\rm w}/_{\rm w}~SM/~StD$	$10\%{\rm ^{w}\!/_w}AE/$ StD	$20\%^{\rm w}/_{\rm w}AE/StD$		
Pre-treatment	162.83 ± 2.73	217.33 ± 4.73	212.83 ± 2.62	201.0 ± 3.27	211.17 ± 3.40	205.0 ± 2.63		
(4 weeks)								
Post-treatment (8	208.17 ± 2.40	171.83 ± 2.18	175.20 ± 2.21 **	$154.67 \pm 2.07^{\ast\ast\ast}$	$157.33 \pm 2.09^{\ast\ast\ast}$	$158.17 \pm 2.09 ***$		
weeks)								
Per cent body	27.84 ± 5.44	-20.93 ± 15.67	$\textbf{-19.19} \pm 6.41$	$\textbf{-23.05} \pm 12.31$	-25.43 ± 11.40	-22.85 ± 5.83		
weight changes (%)	1							

Weight values are expressed as mean \pm SEM. ** p < 0.01;*** p < 0.001 significant body weights difference between the different treatment groups of obese rats given StD supplemented with dried fruit samples and the control groups (1 and 2) of non-obese and obese rats given StD. (One-way ANOVA followed by Tukey post hoc test)

Table 3: Effect of S. macrocarpon and A. esculentus dried fruits ingestion on liver organ weights and histology of liver tissue of rats

Organ weight (g)						
Parameters	Group 1: StD-	Group 2: Obese-StD-	Group 3: Obese-10%	Group 4: Obese-20%	Group 5: Obese-	Group 6: Obese-
	Control	Control	w/w SM/StD	w/w SM/StD	10% ^w / _w AE/StD	$20\%{}^{\rm w}\!/_{\rm w}AE/StD$
Liver weight	2.76 ± 0.14	3.10 ± 0.48	2.58 ± 0.13 **	$2.17 \pm 0.17^*$	$1.86 \pm 0.11^*$	$2.05 \pm 0.15^{*}$
Histology of liver tissue	No histopathological changes seen	No histopathological changes seen	Mild periportal lymphocytic infiltrate and venuous congestion seen	No histopathological changes seen	Mild/moderate periportal inflammation	Mild periportal lymphocytic infiltrate and venuous congestion seen

Weight values are expressed as mean \pm SEM.* p < 0.05; ** p < 0.01 significant liver weights difference between the different treatment groups of obese rats given dried fruit samples and the control groups (1 and 2) of non-obese and obese rats given StD (One-way ANOVA followed by Tukey post hoc test)

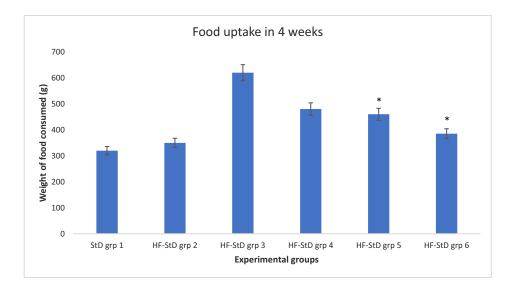


Figure 1: Daily food intake of standard rodent diet (StD) and high-fat diet (HF-StD) by rats.

* p < 0.05 significant difference of feed consumed between the rats given HFD-StD and the rats given StD in control group 1 (One-way ANOVA followed by Tukey post hoc test)

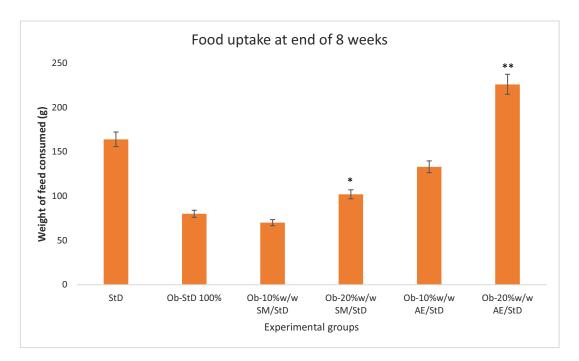


Figure 2: Daily food intake of standard rodent diet (StD) and StD supplemented with *S. macrocarpon* and *A. esculentus* fruits by rats. * p < 0.05; ** p < 0.01 significant difference of feed consumed between the different treatment groups of obese rats fed orally with StD supplemented with dried fruit samples and the control groups (1 and 2) of non-obese and obese rats fed orally with StD (One-way ANOVA followed by Tukey post hoc test).

Parameters	StD	Ob-StD	Ob -10% ^W / _W	Ob-20% ^W / _W	$Ob-10\% W_W$	Ob-20% ^W / _W
			SM / StD	SM / StD	AE / StD	AE / StD
Total Cholesterol (mg/dL)	36.93 ± 4.14	37.83 ± 2.90	34.74 ± 2.75	21.84 ± 2.55 ***	$25.17 \pm 2.12*$	25.68 ± 2.83
High-density lipoprotein (mg/dL)	19.26 ± 2.23	21.42 ± 2.36	18.75 ± 1.68	14.07 ± 1.80	14.28 ± 1.52	13.95 ± 1.68
Low-density lipoprotein (mg/dL)	17.01 ± 2.12	18.37 ± 1.73	11.10 ± 1.06	$4.44 \pm 0.53^{***}$	$7.65 \pm 0.81 \text{**}$	$8.40 \pm 2.04 \text{**}$
Triglycerides (mg/dL)	$12.51\pm\!\!1.82$	15.66 ± 2.95	10.23 ± 1.56	$7.35 \pm 1.35 *$	$5.34\pm0.99^{\boldsymbol{**}}$	$7.38 \pm 1.64 *$
Total bilirubin (mg/dL)	0.61 ± 0.05	0.75 ± 0.05	$0.82\pm0.03^{\boldsymbol{\ast\ast}}$	$0.10 \pm 0.01^{***}$	0.07 ± 0.02 ***	$0.10\pm0.02^{\boldsymbol{\ast\ast\ast\ast}}$
Total protein (g/L)	7.26 ± 0.31	7.58 ± 0.16	$\boldsymbol{6.98 \pm 0.13}$	$6.41\pm0.24\texttt{*}$	6.81 ± 0.18	7.05 ± 0.62
Albumin (g/L)	2.98 ± 0.17	3.03 ± 0.22	2.96 ± 0.06	2.81 ± 0.13	2.66 ± 0.18	2.76 ± 0.09

Table 4: Effect of S. macrocarpon and A. esculentus dried fruits ingestion on rats' serum lipid profile

Values are expressed as mean \pm SEM.* p < 0.05; ** p < 0.01; *** p < 0.001 significant difference of lipid profile between the different treatment groups of obese rats given StD supplemented with dried fruit samples and the control groups (1 and 2) of non-obese and obese rats given StD (One-way ANOVA followed by Tukey post hoc test)

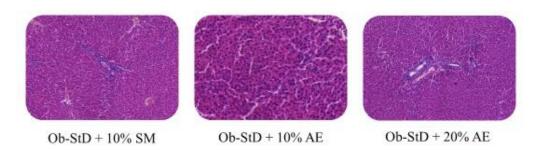


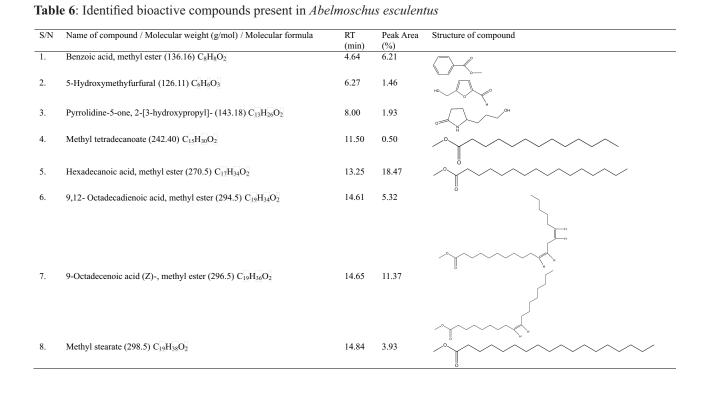
Figure 3: Photomicrographs of the liver of obese rats fed with A: $10\%''_{w}$ *S. macrocarpon* showing mild periportal lymphocytic infiltrate; B: $10\%''_{w}$ *A. esculentus* showing mild/moderate periportal inflammation within the hepatic lobule; C: $20\%''_{w}$ *A. esculentus* showing mild periportal lymphocytic infiltrate.

3.4 Identified phytochemicals

A total of 16 natural compounds were identified from the GC/MS analysis of *S. macrocarpon* L. and *A. esculentus* plant extracts. Tables 5 and 6 show the compounds' molecular weights, molecular formula and retention time with substantial percentage compositions and quality match ranging from 80 to 99% for *S. macrocarpon* and *A. esculentus* plant extracts. The chromatograms are shown in Figures 4 and 5. Six (6) compounds with varying peak areas ranging from 0.5 to 29.63% were commonly identified in both plant samples which include benzoic acid, methyl ester; methyl tetradecanoate; hexadecanoic acid, methyl ester; 9,12- octadecadienoic acid, methyl ester; 9-octadecenoic acid (*Z*)-, methyl ester; and methyl stearate. Other identified compounds found only in *S. macrocarpon* with a peak area range of 0.23 to 1.14% are 2-methoxy-4-vinyl phenol; dodecanoic acid, methyl ester; Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-; 2-naphthalenemethanol,1,2,3,4,4a,5,6.7-octahydro-alpha, alpha, 4a, 8- tetramethyl-; 1H-indene,1-ethylideneoctahydro-7a-methyl-, (1E,3a.alpha.,7a.beta.)-; and 1H-cycloprop[e]azulene,1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl-,[1aR-(1a.alpha.,7. alpha., 7a.beta.,7b.alpha)]. Among the compounds identified in *A. esculentus* are 5-Hydroxymethyfurfural (1.46%) and pyrrolidine-5-one, 2-[3-hydroxypropyl]-(1.93%).

Table 5: Identified bioactive compounds present in Solanum macrocarpon

S/N	Name of compound / Molecular weight (g/mol) / Molecular formula	RT	Peak Area	Structure of compound
		(min)	(%)	
1.	Benzoic acid, methyl ester (136.16) $C_8H_8O_2$	4.63	3.46	
2.	2-Methoxy-4-vinylphenol (150.17) $C_9H_{10}O_2$	7.34	0.23	₩ <u></u>
				<u>}</u> ~
3.	Dodecanoic acid, methyl ester (214.34) $C_{13}H_{26}O_2$	9.57	0.38	
4.	Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-	9.92	1.14	ő
	$(204.35) C_{15}H_{24}$			\sim
5.	2-Naphthalenemethanol,1,2,3,4,4a,5,6.7-Octahydro- alpha,alpha, 4a, 8-	10.76	0.56	YXXX
	tetramethyl- (222.37) $C_{15}H_{26}O$			
6.	1H-Indene, 1-ethylideneoctahydro-7a-methyl-, (1E,3a.alpha.,7a.beta.)- (164.29)	10.96	0.88	\sim
	$C_{12}H_{20}$			$\langle U \rangle$
7.	Methyl tetradecanoate (242.40) $C_{15}H_{30}O_2$	11.50	1.08	~~~~~
8.	1H-Cycloprop[e]azulene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl-,	12.44	1.03	
0.	$[1aR-(1a.alpha.,7.alpha.,7a.beta.,7b.alpha)]$ (204.35) $C_{15}H_{24}$	12.44	1.05	$\langle \uparrow \rangle$
	[1ak-(1a.aipha.,7.aipha.,7a.octa.,70.aipha)] (204.55) C151124			
9.	Hexadecanoic acid, methyl ester (270.5) C ₁₇ H ₃₄ O ₂	13.25	29.63	
				0
10.	Hexadecanoic acid, ethyl ester (284.5) $C_{18}H_{36}O_2$	13.80	0.42	
11.	9,12- Octadecadienoic acid, methyl ester (294.5) $C_{19}H_{34}O_2$	14.61	8.86	ö
	(2) (3) (2) (3) (2) (3) (2) (3) (3) (3) (3)	1	0.00	
12.	0 October and (7) method actor (20(5) C H O	14.66	24.35	1
12.	9-Octadecenoic acid (Z)-, methyl ester (296.5) $C_{19}H_{36}O_2$	14.00	24.55	
				4
13.	Methyl stearate (298.5) C ₁₉ H ₃₈ O ₂	14.84	4.57	
14.	Eicosanoic acid, methyl ester (326.6) $C_{21}H_{42}O_2$	16.46	0.44	ő
	Eleosanole acid, methyl ester (520.0) C211142O2			



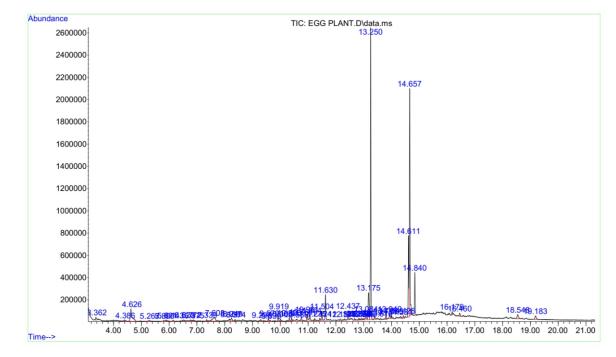


Figure 4: GC/MS chromatogram of Solanum macrocarpon

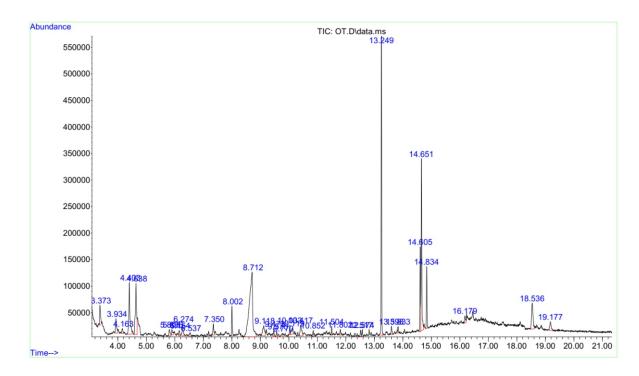


Figure 5: GC/MS chromatogram of Abelmoschus esculentus

4. Discussion

Excessive intake of calories contributes to the development of obesity. Daily consumption of healthy food is considered important to prevent obesity and other obesity-induced health problems such as type 2 diabetes, cancer and cardiovascular diseases among others ²⁶. In this study, the preventive effect of S. macrocarpon (African eggplant) and A. esculentus (okra) on weight gain and appetite was evaluated with an in vivo obesity model represented by high-fat diet- (HF-StD) induced obese rats. Prior to supplementation of the animal standard feed with S. *macrocarpon* and A. esculentus plant samples, a percentage increase in body weights of rats in the HF-StD-fed group was observed as compared to the rats in the StD-control group. The percentage increase in body weights of rats observed in the HF-StD-fed groups that commensurate with increased total food intake indicates that the HF-StDinduced obesity model was successfully established in the Wistar rats, which is in agreement with previous studies 27,28,29 After an 8-week post-treatment period with a standard diet supplemented with varying percentages of S. *macrocarpon* and A. esculentus plant samples, there was a reduction in rats' body and liver weights that coincides with decreased total food intake in all HF-StD-fed groups given standard feed and feed supplemented with the plant

samples. The supplementation of diets with *S*. *macrocarpon* and *A*. *esculentus* plant samples affected food intake by rats, indicating the possibility of appetite suppression as an anti-obesity mechanism involved in the observed reduction of rat weights. Body weight increases with excessive intake of calories and when energy consumption exceeds energy expenditure ^{23,30}. Therefore, the observed reduction in rats' body weight gain in Ob-Std-supplemented with plant sample groups may be attributed to the consumption of *S. macrocarpon* and *A. esculentus* as no reduction in the body weights of rats in the StD-control group was evident.

It has been established that a high-fat diet (HF-StD) and obesity cause non-alcoholic fatty acid liver diseases resulting in the accumulation of triglycerides (TG) in the liver ^{29,31}. It was demonstrated in this study that with the consumption of a diet supplemented with either *S. macrocarpon* or *A. esculentus*, the liver weights of rats were significantly lowered as compared to the Ob-StD-control and StD-control groups. More so the hepatic tissue histology did not show major pathological changes.

The levels of major serum lipids parameters such as TCHO, HDL-c, LDL-c, TG, and TBIL were measured after the 8 weeks of treatment of obese rats with dried fruit samples of *S. macrocarpon* and *A. esculentus*. The observed increase in the levels of TCHO, HDL-c, LDL-c, TG, and TBIL in the Ob-StD group compared to the Ob-StD+plant sample (treatment) groups may be due to an initial consumption of HF-StD by the rats that may have caused an increase in free fatty acids, which are raw materials for the production of TCHO, LDL-c, HDL-c and TG^{31,32}. However, results from this study revealed that the administration of standard feed (StD) supplemented with dried fruit samples of *S. macrocarpon* and *A. esculentus* (Ob-StD+SM and Ob-StD+AE) significantly decreased levels of the lipid profile of obese rats as compared to the two control groups. The observed result may be attributed to the phytoconstituents of *S. macrocarpon* and *A. esculentus*.

Phytochemical analysis of Solanum macrocarpon L. and Abelmoschus esculentus plant extracts via GC/MS technique revealed the presence of phenolic acid derivatives such as benzoic acid; saturated fatty acids such as hexadecenoic acid methyl ester; monounsaturated fatty acids such as 9-octadecenoic acid; and polyunsaturated fatty acids such as 9,12-octadecadienoic acid. Phenolic compounds act as natural antioxidants that exert therapeutic effects such as antidiabetic, and antiinflammatory as well as prevent various forms of obesityinduced diseases ^{33,34,35}. Long-chain polyunsaturated fatty acids have been reported to increase the release of satiety hormones such as cholecystokinin which delays gastric emptying and produces an increased feeling of satiety and decreased appetite 36,37. These results suggest that the observed reduction of lipid profiles as well as reduction in rats' body and liver weights that coincides with decreased total food intake might be attributed to the effects of these compounds and others identified in S. macrocarpon and A. esculentus fruits.

5. Conclusion

The potential of *Solanum macrocarpon* (African eggplant) and *Abelmoschus esculentus* (okra) in the management of high-fat diet-induced increase in body and liver weights and regulation of serum lipid profiles has been established. Phytochemical analysis revealed the presence of phenolic acid derivatives and fatty acids that may have contributed to the observed effects. *S. macrocarpon* and *A. esculentus* are known for their nutritional values and people usually consume these fruits. The study demonstrates the potential use of these fruits for the prevention and management of obesity. Further investigations will be required to determine the involvement of obesity-related hormones such as plasma adiponectin, leptin and insulin in the mechanisms of anti-obesity activity of these fruits.

Acknowledgements

The authors express immense gratitude to Mr. Sunday Adenekan, Department of Biochemistry, Mr. Micah Chijioke, Department of Pharmacology, therapeutics and toxicology, and Mr. Farouk Olatunji of APIN Laboratory, Central Research Laboratory, of the College of Medicine, University of Lagos for the technical assistance provided.

References

- Nderitu KW, Mwenda NS, Macharia NJ, Barasa SS, Ngugi MP. Antiobesity Activities of Methanolic Extracts of *Amaranthus dubius*, *Cucurbita pepo*, and *Vigna unguiculata* in Progesterone-Induced Obese Mice. Evid Based Complement Alternat Med. 2017;2017:4317321. doi: 10.1155/2017/4317321. Epub 2017 Aug 30. https://doi.org/10.1155/2017/4317321.
- Kim HJ, Koo KA, Park WS, Kang DM, Kim HS, Lee BY, Goo YM, Kim JH, Lee MK, Woo DK, Kwak SS, Ahn MJ. Anti-obesity activity of anthocyanin and carotenoid extracts from colorfleshed sweet potatoes. J Food Biochem. 2020 Aug 18:e13438. doi: 10.1111/jfbc.13438. Epub ah e ad of print. PMID: 32812262. https://doi.org/10.1111/jfbc.13438.
- 3. WHO 2008 Waist circumference and waist-hip ratio: report of a WHO expert consultation. 8–11 December. Geneva, Switzerland: www.who.int/nutrition/publication/obesity/WH O_report_waistcircumference_and_waisthip_ration/en/
- 4. World Obesity Federation, World Obesity Atlas 2 0 2 3 . <u>https://data.worldobesity.org/publications/?cat=1</u> <u>9</u>.
- World Health Organization. "Obesity and Overweight. <u>https://www.who.int/newsroom/fact sheets/detail/obesity-and-overweight</u>. 9 June 2021.
- Mubarak A, Hodgson JM, Considine MJ, Croft KD, Matthews VB. Supplementation of a high-fat diet with chlorogenic acid is associated with insulin resistance and hepatic lipid accumulation in mice. J Agric Food Chem. 2013 May 8;61(18):4371-8. doi: 10.1021/jf400920x. Epub 2013 Apr 26. Erratum in: J Agric Food Chem.

2015 Feb 18;63(6):1882.

- Qin N, Chen Y, Jin MN, Zhang C, Qiao W, Yue XL, Duan HQ, Niu WY. Anti-obesity and anti-diabetic effects of flavonoid derivative (Fla-CN) via microRNA in high fat diet induced obesity mice. Eur J Pharm Sci. 2016 Jan 20;82:52-63. doi: 10.1016/j.ejps.2015.11.013. Epub 2015 Nov 18.
- 8. Yun JW. Possible anti-obesity therapeutics from nature--a review. Phytochemistry. 2010 O c t; 7 1 (14-15): 1625-41. doi: 10.1016/j.phytochem.2010.07.011. Epub 2010 Aug 21.
- Zhang JY, Xiao X, Dong Y, Zhou XH. Antiobesity Action of Fermented Barley Extracts with Lactobacillus plantarum dy-1 and Associated MicroRNA Expression in High-fat Diet-induced Obese Rats. Biomed Environ Sci. 2019 Oct;32(10):755-768. doi: 10.3967/bes2019.095
- Joffe M, Robertson A. The potential contribution of increased vegetable and fruit consumption to health gain in the European Union. Public Health Nutr. 2001 Aug;4(4):893-901. doi: 10.1079/phn2001126.
- Mellendick K, Shanahan L, Wideman L, Calkins S, Keane S, Lovelady C. Diets Rich in Fruits and Vegetables Are Associated with Lower Cardiovascular Disease Risk in Adolescents. Nutrients. 2018 Jan 27;10(2):136. doi: 10.3390/nu10020136.
- Gomathi D, Kalaiselvi M, Ravikumar G, Devaki K, Uma C. GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of Evolvulus alsinoides (L.) L. J Food Sci Technol. 2015 Feb;52(2):1212-7. doi: 10.1007/s13197-013-1105-9. Epub 2013 Jul 24.
- Burkill HM. The Useful Plants of West Tropical A frica, vol. 1. Royal Botanic Gardens, Kew, (1985). pp. 252–258.
- Oboh G, Ekperigin MM, Kazeem MI. Nutritional and haemolytic properties of eggplants (Solanum macrocarpon) leaves Journal of Food Composition and Analysis. 2005 ;18(2-3):153-160. DOI: 10.1016/j.jfca.2003.12.013.
- Famuwagun AA, Taiwo KA, Gbadamosi SO, Oyedele DJ, Aluko RE and Adebooye OC (2017). Extraction Optimization and Antioxidant Properties of African Eggplant (Solanum macrocarpon) Leaf Polyphenols. Hindawi Journal of Food Quality 2017 (2159183): 1-14.

https://doi.org/10.1155/2017/2159183.

- Bello SO, Muhammad BY, Gammaniel KS, Abdu-Aguye I, Ahmed H, Njoku C, Pindiga UH, Salka AM. Preliminary evaluation of the toxicity and some pharmacological properties of the aqueous crude extract of *Solanum melongena*. *Research Journal of Agriculture, Biology and Science* 2005;1(1): 1-9
- 17. Emiloju OC, Chinedu SN (2016) Effect of Solanum aethiopicum and Solanum macrocarpon fruits on weight gain, blood glucose and liver glycogen of Wistar rats. World Journal of Nutrition and Health 2016;4(1):1-4.http://pubs.sciepub.com/jnh/4/1/1
- Durazzo A, Lucarini M, Novellino E, Souto EB, Daliu P, Santini A. Abelmoschus esculentus (L.): Bioactive Components' Beneficial Properties-Focused on Antidiabetic Role-For Sustainable Health Applications. Molecules. 2018 Dec 21;24(1):38. doi: 10.3390/molecules24010038.
- Dantas TL, Buriti FCA, Florentino ER. Okra (Abelmoschus esculentus L.) as a potential Functional Food Source of Mucilage and Bioactive Compounds with Technological Applications and Health Benefits. Plants 2021;10: 1683. https://doi.org/10.3390/plants10081683.
- 20. Islam MT. Phytochemical information and pharmacological activities of Okra (*Abelmoschus esculentus*): A literature-based review. Phytother Res. 2019 Jan;33(1):72-80. doi: 10.1002/ptr.6212. Epub 2018 Oct 22.
- Haque MA, Hossain MS, Sayed NMA, Islam MT, Khan MR, Ahmmed F, Zohora FT, Ağagündüz D, Ming LC, Capasso R. *Abelmoschus esculentus* (L.) Moench Pod Extract Revealed Antagonistic Effect against the Synergistic Antidiabetic Activity of Metformin and Acarbose upon Concomitant Administration in Glucose-Induced Hyperglycemic Mice. *Biologics*. 2022; 2(2):128-138. https://doi.org/10.3390/biologics2020010
- National Academy of Sciences. Guide for the Care and Use of Laboratory Animals, 8th edition. ed. National Academies Press, Washington DC; 2011.
- 23. Morales-Avila UM, Becerra-Verdin EM, Guadalupe Sayago-Ayerdo, S., Tolman, J.P., Montalvo-Gonzalez, E. Anti-obesity and hepatoprotective effects in obese rats fed diets supplemented with fruit purees. *Food and Science Technology* 2020;40:33-41

- Ibrahim MB, Sowemimo AA, Sofidiya MO, Badmos KB, Fageyinbo MS, Abdulkareem FB, Odukoya OA. Sub-acute and chronic toxicity profiles of Markhamia tomentosa ethanolic leaf extract in rats. J Ethnopharmacol. 2016 Dec 4;193:68-75. doi: 10.1016/j.jep.2016.07.036. Epub 2016 Jul 15.
- Olivia, N.U., Goodness, U.C. & Obinna, O.M. Phytochemical profiling and GC-MS analysis of aqueous methanol fraction of *Hibiscus asper* leaves. *Futur J Pharm Sci* 2021;7, 59. <u>https://doi.org/10.1186/s43094-021-00208-4</u>
- Arya A, Nahar L, Khana HU, Sarker SD. Antiobesity natural products. In *Annual Reports in Medicinal Chemistry 2020*;55:411-433).
 A c a d e m i c P r e s s . https://doi.org/10.1016/bs.armc.2020.02.006
- 27. Bussey CE, Withers SB, Aldous RG, Edwards G and Heagerty A Bussey CE, Withers SB, Aldous RG, Edwards G, Heagerty AM. Obesity-Related Perivascular Adipose Tissue Damage Is Reversed by Sustained Weight Loss in the Rat. Arterioscler Thromb Vasc Biol. 2016 Jul;36(7):1377-85. doi: 10.1161/ATVBAHA.116.307210. Epub 2016 May 12.
- 28. Han Q, Yeung SC, Ip MSM, Mak JCW. Dysregulation of cardiac lipid parameters in highfat high-cholesterol diet-induced rat model. Lipids Health Dis. 2018 Nov 14;17(1):255. doi: 10.1186/s12944-018-0905-3.
- 29. Cho BO, Che DN, Shin JY, Kang HJ, Kim JH, Jang SI. Anti-obesity effects of enzyme-treated celery extract in mice fed with high-fat diet. J Food Biochem. 2020 Jan;44(1):e13105. doi: 10.1111/jfbc.13105. Epub 2019 Dec 1.
- 30. Jambocus NGS, Ismail A, Khatib A, Mahomoodally F, Saari N, Mumtaz MW, Hamid AA. *Morinda citrifolia* L. leaf extract prevent weight gain in Sprague-Dawley rats fed a high fat diet. Food Nutr Res. 2017 Jul 27;61(1):1338919. doi: 10.1080/16546628.2017.1338919.
- Ferolla SM, Silva LC, Ferrari Mde L, da Cunha AS, Martins Fdos S, Couto CA, Ferrari TC. Dietary approach in the treatment of nonalcoholic

fatty liver disease. World J Hepatol. 2015 Oct 28;7(24):2522-34. doi: 10.4254/wjh.v7.i24.2522.

- 32. Mejia de Grubb MC, Levine RS, Zoorob RJ. Diet and Obesity Issues in the Underserved. Prim Care.
 2 0 1 7 M a r ; 4 4 (1) : 1 2 7 1 4 0 . d o i : 10.1016/j.pop.2016.09.014. Epub 2017 Jan 2.
- 33. Shahidi F, Ambigaipalan P. Phenolics and polyphenolics in foods, beverages and spices: antioxidant activity and health effects A review. *Journal of Functional Food* 2015. 18
 Part B: 820-897. https://doi.org/10.1016/j.jff.2015.06.018
- 34. Gonçalves S, Moreira E, Grosso C, Andrade PB, Valentão P, Romano A. Phenolic profile, antioxidant activity and enzyme inhibitory activities of extracts from aromatic plants used in Mediterranean diet. J Food Sci Technol. 2017 Jan;54(1):219-227. doi: 10.1007/s13197-016-2453-z. Epub 2016 Dec 26.
- 35. Ibrahim M, Oyebanji E, Fowora M, Aiyeolemi A, Orabuchi C, Akinnawo B, Adekunle AA. Extracts of endophytic fungi from leaves of selected Nigerian ethnomedicinal plants exhibited antioxidant activity. BMC Complement Med Ther. 2021 Mar 20;21(1):98. doi: 10.1186/s12906-021-03269-3.
- Lawton CL, Delargy HJ, Brockman J, Smith FC, Blundell JE. The degree of saturation of fatty acids influences post-ingestive satiety. Br J Nutr. 2000 May;83(5):473-82.
- 37. Arika WM, Kibiti CM, Njagi JM, Ngugi MP. Antiobesity effects of dichloromethane leaf extract of *Gnidia glauca* in high fat diet-induced obese rats. Heliyon. 2019 Nov 21;5(11):e02800. doi: 10.1016/j.heliyon.2019.e02800.