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Original Research Article

Antiobesity Activity of Extract, Fractions and Pure Compounds from Husk of *Zea mays*

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ABSTRACT

Zea mays L. (Poacae) husk is used in Ibibio traditional medicine for the treatment of various ailments including diabetes mellitus, dyslipidemia and malaria. The aim of this study was to evaluate the husk extract and fractions for obesity preventive activity in rats fed on high fat diet (HFD) to validate its folkloric claims. The ethanol husk extract of *Zea mays* (187-561 mg/kg) and fractions (hexane, dichloromethane (DCM), ethyl acetate(EA) and methanol, 200 mg/kg) were investigated for *in vivo* antiobesity activity in HFD-fed rats for 42 day and *in vitro* inhibitory activity against porcine pancreastic lipase. Orlistat and cetilistat were used as standard drugs. Body weights, body mass index, antherogenic index, lipid profile, blood glucose level, liver function indices and antioxidant biomarkers were parameters measured. Isolation and identification of compounds from active fraction was carried out by column chromatography and Nuclear Magnetic Resonance (NMR) spectroscopy, respectively. The isolated compounds were tested for porcine pancreatic lipase inhibitory activity. The husk extract and fractions caused considerable reduction of serum lipid levels, body and organ weights, antherogenic indices, Blood glucose level, liver function parameters and also increased antioxidative markers levels with the DCM fraction exerting the highest activity. Stigmasterol and stigmasterylpalmitate isolated from DCM fraction demonstrated inhibitory activity against porcine pancreatic lipase. These results suggest that the husk extract of *Zea mays*possesses obesity preventive potentials which lay credence to its usage in folkloric medicine in the treatment of dyslipidemia and related diseases.

Keywords: *Zea mays*, Antiobesity, Stigmasterol, Stigmasterylpalmitate, Stigmasteryl stearate, High fat diet.

Introduction

Obesity is a metabolic disorder caused by an imbalance in body energy regulation and characterised by abnormal or excessive fat accumulation that poses a risk to health.[1] Obesity is a public health problem assuming serious epidemic status and prevalence globally.[2]it is commonly associated with coronary heart disease, dyslipidemia, glucose intolerance, diabetes, hypertension and some cancers.[3]Lack of exercise, sedentary lifestyles and the consumption of energy rich diets are predisposing factors to the development of obesity.[4] Due to unsatisfactory treatment outcome with conventional drugs in the management of obesity, plants and other natural products used in traditional medicine are being investigated for possible antiobesity activity. *Zea mays*L.(Poacae) also called maize or corn, is a grass and food plant cultivated for human and animal benefits. The plant is tall and bears ears that are enclosed in modified leaves known as husks.[5] In addition to its nutritive values, various parts of the plants are used in ethnomedicine for the treatment of several ailments such as diabetes and dislipidemia,[6,7] inflammatory diseases,[8]pains and arthritis[9] and ulcer.[10]

Reported pharmacological properties of the husk extract include; analgesic, anti-inflammatory,[9] antioxidant,[11] antidepressant,[8] antimalarial and antiplasmodial,[7] hepatoprotective,[12,13,14,] antidiabetic and hypolipidemic[15] and nephroprotective,[16,17] antiulcer[18] activities. The median lethal dose (LD_{50}) of the ethanol husk extract was determined to be 1874.83 mg/kg.[8]Phenolic compounds (gallic acid, protocatechuic acid, chlorogenic acid, cafeic acid, femlic acid, rutin, resveratrol, and kaempferol)[11] and anthocyannins[19]have been reportedly isolated from the husk extract. The study reports obesity preventive activity of the husk extract and fractions as well as isolation and characterisation of pure compounds with pancreastic lipase inhibitory activity from *Zea mays* husk to validate the ethnobotanical usage of the corn husk in the treatment of dyslipidemia.

Materials and methods

Collection of plant materials

Fresh husks of *Zea mays* were collected from a farmland in Uruan LGA, AkwaIbom State, Nigeria in August 2020. The husks were identified and authenticated as *Zea mays* by Prof. Margaret Bassey, a taxonomist in the Department of Botany and Ecological studies, University of Uyo, Uyo, Nigeria. Herbarium specimen (FPHUU, 614) was deposited at the Faculty of Pharmacy Herbarium, University of Uyo, Uyo.

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Extraction
Fresh husk of *Zea mays* were washed, cut into smaller pieces and dried under shade for two weeks. The husks were pulverized to powder using electric grinder. The powdered leaves material was divided into two parts; one part (1.5 kg) was macerated in 50% ethanol (7.5 L) for 72 hours at room temperature (28 ± 2°C), while the other part (1.5 kg) was successively macerated for 72h in each of these solvents (2 x 5 L), n-hexane, dichloromethane, ethyl-acetate and methanol to give corresponding fractions of these solvents. The fractions were thereafter filtered and the filtrates were concentrated and evaporated to dryness in *vacuo*at 40°C using a rotary evaporator (Buchi Lab, Switzerland). The extract and fractions were stored in a refrigerator, until used for the proposed experiments.

Isolation and Purification of compounds from dichloromethane fractions of Zea mays husk
The dichloromethane fraction (12 g) which was found to be active during the experiment was subjected to silica gel column chromatography (Merck, 60-120 mesh) and gradient-eluted with *n*-hexane containing increasing quantity of dichloromethane, followed by increasing quantity of ethyl acetate and methanol. Eluates (20 mL each) were collected, monitored on silica TLC plates (Merck, Germany) in hexane: DCM: EA (2:1:1) using vanillin-sulphuric acid as spray reagent. Sixty fractions were obtained and bulked together based on their similar TLC characteristics (Rf values, colour reaction with spray reagents) to give four semi-pure residues coded F1 – F4. Fractions F1, F2 and F4 were small in quantity to be purified further and were discarded. Based on purity on TLC and quantity, bulked fraction F3 was further purified on silica gel by column chromatography using *n*-hexane in increasing polarity with dichloromethane and ethyl acetate (1:1, 1:1.5 and 1:2) while purity was monitored with TLC (dichloromethane and ethyl acetate) (3:1) to give three semipure compounds coded S1, S2, and S3. These three compounds were further purified using silica gel column chromatography to obtain pure compounds; HS1 (11.0 mg), HS2 (8.24 mg) and HS3 (12.03 mg). The chemical structures of isolated pure compounds were elucidated using NMR spectroscopy.

NMR spectroscopy
The ^{1}H- and ^{13}C-NMR spectra for the isolated compounds (HS1, HS2 and HS3) were obtained on a Bruker 400 and 100 MHz instrument, respectively. Chemical shifts were reported in δ (ppm) using the solvent ($CDCl_3$) and coupling constants (J) were measured in Hertz.

Animals
Wistar male rats (151 – 162 g) used for the experiments were gotten from Animal house of Department of Pharmacology and Toxicology, University of Uyo. The animals were housed in standard cages and were maintained on a standard pelleted feed (Guinea feed) and water *ad libitum*. Approval for animal studies protocols were obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo (UU/CHS/AE/21/023).

Experimental design and animal treatment
The experiment lasted for 6 weeks according to modified method of Nazish *et al*.,[20] using 60 male Wistar rats with initial body weight of approximately 153.15 ± 36.95 g. In this study, rats were given a hypercaloric diet containing 46% commercial food (Guinea Feed), 46% condensed milk and 8% corn oil[22] to induce obesity. Diets (1.0 kg) were prepared weekly and stored in a refrigerator. The animals were fed the hypercaloric diet and the following treatments for six weeks orally: Group I (normal control) consisted of animals fed with commercial chow (normal grower's Feed). Group II was fed with a high-fat control diet containing 46% commercial Feed (Guinea Feed), 46% condensed milk and 8% corn oil.[21] Group III was fed with a high-fat diet plus 187 mg/kg body weight of *Z. mays* husk extract. Group IV was fed with a high-fat diet plus 374 mg/kg BW of *Zea mays* husk extract. Group V was fed with a high-fat diet plus 561 mg/kg BW of *Z. mays* husk extract. Group VI was fed with a high-fat diet plus 374 mg/kg BW of hexane fraction of *Z. mays* husk extract. Group VII was fed with a high-fat diet plus 374 mg/kg BW of DCM fraction of *Z. mays* husk extract, Group VIII was fed with a high fat diet plus 374 mg/kg BW of ethyl acetate fraction of *Z. mays* husk extract, Group IX was fed with a high fat diet plus 374 mg/kg BW of methanol fraction of *Z. mays* husk extract and Group X was fed with a high-fat diet plus Orlistat 5 mg/kgBW.
Each treatment was administered daily, diluted with water, and the administration was performed by gavage, using sterilized stainless steel cannula. The treatment lasted for 42 days.

Collection of blood samples and organs
After 42 days of treatment (24 hours after the last treatment), the rats were weighed again and sacrificed under light diethyl ether vapour following overnight fast. Blood samples were collected by cardiac puncture and used immediately. Blood were collected into plain centrifuge tubes and centrifuged immediately at 1500 rpm for 15 min to separate the serum at room temperature to avoid haemolysis. This was used for biochemical assays.

Determination of the effect of the husk crude extract and fractions on the lipid profile of the treated obese rats
Serum total cholesterol, triglyceride and high density lipoprotein (HDL) levels of the obese rats were measured by enzymatic colorimetric methods using Randox diagnostic kits. The low and very low-density lipoprotein (LDL and VLDL) were estimated from the formula of Friedwald *et al.*[22]

Evaluation of the effect of the husk extract and fractions on some biochemical parameters of liver of HFD-induced obese rats
Serum was separated from the blood of each animal sacrificed and the sera were stored in the refrigerator until used for biochemical determinations such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphotase (ALP), and total bilirubin. The determinations were spectrophotometrically done using Randox analytical kits according to standard procedures of manufacturer's protocols.[23]
The livers of the animals were surgically removed, weighed and washed with ice cold 0.9% NaCl and homogenates were made in a ratio of 1 g of wet tissue to 9 mL of 1.25% KCl by using motor driven Teflon-pestle. The homogenates were centrifuged at 7000 rpm for 10 min at 4˚C and the supernatants were used for the assays of superoxide dismutase (SOD),[24] catalase (CAT),[25] glutathione peroxidase (GPx)[26] and reduced glutathione (GSH).[27]

Anthropometric measurements
The body weights were determined once a week. Body mass index (BMI) was calculated from formula:

$$\text{BMI} = \frac{\text{Body weight (g)}}{\text{Length}^2\,(\text{cm}^2)}$$

The atherogenic index (AI) was calculated using the formula of Aziz *et al*.,[28]

$$\text{AI} = \frac{TC - HDL - C}{HDL - C}$$

Pancreatic lipase inhibitory activity
Evaluation of pancreatic lipase inhibitory activity was performed by measuring the release of 4-methylumbelliferone (4MUF) from the substrate 4-methylumbelliferyl oleate (4MUFO) as reported by Bitou *et al.*[29]with slight modifications. Briefly, 50 µL of the test samples (HS1, HS 2 and HS3) and 50 µL of pancreatic lipase (100 µg/ml in phosphate buffer of 0.2 M, pH 7.4) were mixed and incubated at room temperature for 10 min. Afterward, 100 µL of the substrate 4MUFO (0.5 mM) was added. After 10 min, the fluorescence from the release of 4MUF was measured using a microplate reader with excitation and emission wavelengths of 355 and 460 nm, respectively. Cetilistat was used as a positive control. The percentage of pancreatic lipase inhibitory activity was calculated using following equation:

$$\text{Inhibition (\%)} = [1 - (As / Ac) \times 100],$$

Where As and Ac are the absorbances of sample and control, respectively. From these data, curve was plotted and inhibitory concentration (IC_{50}) value was calculated, which is defined as the concentration (µg/ml) of the compounds required for 50% inhibition of enzyme.

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Statistical analysis
Data were analysed statistically using one-way ANOVA followed by Tukey-Kramer multiple comparison test. Differences between means were considered significant at 5% level of significance i.e. $p \leq 0.05$.

Results and Discussion

Effect of ethanol husk extract and fractions of Zea mays on the weights of HFD- induced obese rats.
Administration of husk extract and fractions of *Zea mays* on HFD-induced obese rats for 42 days demonstrated dose-dependent and considerable reductions in the body weights of the rats. These reductions were significant (p<0.05-0.01) when compared with the obese control. DCM fraction, followed by the highest dose of the extract (561 mg/kg) and ethyl acetate fraction were found to exert the highest effects. The DCM fraction was found to have comparable activity to that of Orlistat (5 mg/kg) (Table 1).

Effect of husk extract and fractions of Zea mays on organ weights of HFD-induced obese rats
Treatment of HFD –induced obese rats with husk extract and fractions of *Zea mays* did not caused significant (p>0.05) decreases in the weights of liver and kidneys of the obese rats compared to obese control except the DCM fraction and orlistat groups (p<0.05) exerting the highest reductions (Table 2).

Hypolipidemic effect of husk extract and fractions on HFD- induced obese rats
Administration of husk extract and fractions of *Zea mays* to obese rats caused a dose-dependent and significant reductions (p<0.05-0.001) in the levels of total cholesterol, triglyceride, LDL and VLDL with the DCM fraction exerting the highest effect followed by ethyl acetate fraction. The effect of the highest dose of the extract (561 mg/kg) as well as that of DCM fraction was comparable to that of Orlistat in some cases. The husk extract and fractions also caused prominent increases in HDL levels of the extract/ fractions-treated groups with the highest dose and DCM fraction having the highest effect. These increases were significant (p<0.05) when compared to obese control group (Table 3).

Effect of husk extract and fractions on the atherogenic index
The atherogenic index was found to be significantly higher in the HFD group as compared to that of the normal control group. However, administration of the husk extract and fractions significantly reduced the elevated atherogenic index in a dose-dependent fashion with DCM treated group having the lowest index.

Effect of husk extract and fractions on liver function parameters of HFD rats.
Administration of HFD to rats for 42 days caused significant (p < 0.001) elevation in the level of AST, ALT, ALP and total bilirubin when compared to control. However, treatment of the HFD-induced obese rats with husk extract and fractions of *Zea mays* caused significant (p<0.05) reductions in the level of total bilirubin, ALT, ALP and AST when compared to control with the highest dose of the extract (561 mg/kg) having the highest activity followed by n-hexane and dichloromethane fractions. The Orlistat, group was found to have a high level of these liver parameters when compared to control (Table 4).

Effect of husk extract and fraction on Liver antioxidant enzymes of HFD-fed rats
Administration of HFD to rats for 42 days caused significant (p<0.01-0.001) decrease in antioxidant enzymes (SOD, CAT, GPX) and GSH levels with corresponding significant (p<0.001) increase in MDA levels when compared to normal control. Treatment of HFD-fed rats with corn husk extract and fractions caused significant (p<0.05-0.001) dose-dependent elevation in the levels of the antioxidant enzymes (SOD, CAT, GPX) when compared to HFD control. Similarly, GSH level was significantly (p<0.001) elevated, while MDA level was significantly (p<0.05-0.001) decreased following treatment with the husk extract and fractions when compared to HFD control. Similar elevations of the enzymes and GSH levels as well as reduction in MDA level were observed with Orlistat(Table 5).

In vitro porcine pancreas lipase inhibitory effect
The inhibitory activities of the isolated compounds (HS1, HS2 and HS3) against porcine pancreatic lipase are shown in Table 4. HS2 had the highest inhibitory effect with IC_{50} of 14.36 ± 0.71 µg/mL followed by HS1 with IC_{50} of 18.55 ± 1.08 µg/mL, while HS3 had no effect on the lipase enzyme. However, the standard was found to have a more significant inhibition with IC_{50} of 7.17 ± 0.54µg/mL (Table 6)

Identification of isolated compounds
HS1:StigmasterolWhite crystal; m.p. 168.0–170.0∘C; 1H-NMR (400MHz, $CDCl_3$): δ5.34 (d, 1H, J = 4.6Hz, H-6), 5.16 (m, 1H, H-22), 5.03 (m, 1H, H- 23), 3.53 (m, 1H, H-3), 1.00 (s, 3H, H-19), 0.916 (d, 1H, J = 5.75Hz, H-21), 0.829 (m, 9H,H-26, H-27,H-29), 0.685 (s, 3H, H-18); 13C NMR (125MHz, $CDCl_3$): δ140.8 (C-5), 138.4 (C- 20), 129.4 (C-21), 121.8 (C-6), 71.9 (C-3), 56.9 (C-14), 56.2 (C- 17), 51.3 (C-22), 50.2 (C-9), 42.4 (C-4), 42.3 (C-13), 39.9 (C- 12), 39.8 (C-18), 37.4 (C-1), 36.6 (C-10), 32.0 (C-2), 31.7 (C-7), 29.2 (C-8), 29.0 (C-16), 28.3 (C-25), 25.5 (C-23), 24.4 (C-15),21.2 (C-11), 19.9 (C-27), 19.5 (C-26), 19.1 (C-19), 18.9 (C-28), 21.2 (C-11), 19.9 (C-27), 19.5 (C-26), 19.1 (C-19), 18.9 (C-28), 12.3 (C-29), 12.1 (C-24).These data are in agreement with those reported in literature.[30,31]
HS 2: Stigmasteryl Palmitate.1HNMR($CDCl_3$): δ5.36 (1H, m, H-6) 5.20 (1H, m, H-22),5.05 (1H, m, H-23), 3.54 (1H, brs, H-3α), 2.33 (2H, m, H2-21),1.27 (26H, brs, 13 × CH2), 1.02 (3H, brs, Me-19), 0.95 (3H, d,J = 6.5Hz,Me-21), 0.89 (3H, d, J = 6.5Hz,Me-26), 0.87 (3H,d, J = 6.2Hz, Me-27), 0.84 (3H, t, J = 6.6Hz, Me-16), 0.82(3H, d, J = 6.1Hz, Me-29), 0.69 (3H, brs, Me-18).255.These data are in agreement with those reported in literature.[30,31]

Table 1: Effect of ethanol husk extract and fractions of *Zea mays* on the weights of HFD- induced obese rats

Treatment	Dose (mg/kg)	Weights of Rats (g)				BMI
		Week 1	Week 6	Week 12	% Increase in body weight	Body Mass Index (Body weight/Length^2)
Normal Control	10 mL/kg	152.0 ± 7.66	172.0 ± 19.24	201.4 ± 25.0	32.50	0.51
Obese control	-	154.8 ± 10.05	194.4 ± 16.54	255.3 ± 18.45	64.92	0.78
Orlistat	5	152.6 ± 11.18	172.5 ± 17.55	215.3 ± 58.76^c	41.08	0.59
Crude extract	187	154.5 ± 8.37	183.6 ± 19.78	235.6 ± 18.09	52.49	0.68
	374	151.4 ± 12.35	180.25 ± 11.65	228.5 ± 25.28^a	50.92	0.60
	561	150.8 ± 15.58	173.25 ± 27.37	218.7 ± 31.20^c	45.02	0.56
n- hexane fraction	374	152.6 ± 9.52	178.3 ± 20.92	237.0 ± 24.61$^{a\backslash}$	55.30	0.69
Dichloromethane fraction	374	158.68 ± 8.74	170.75 ± 12.98	221.25 ± 10.84^b	39.43	0.58
Ethyl acetate fraction	374	154.5 ± 14.26	181.33 ± 15.60	228.33 ± 16.02^c	47.78	0.63
Methanol fraction	374	158.22 ± 10.45	179.66 ± 13.17	239.33 ± 15.16	51.26	0.66

Data are expressed as MEAN ± SEM, Significant at ^{a}p<0.05, ^{b}p< 0.01, ^{c}p< 0.001, when compared to obese control. (n = 6).

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HS 3: Stigmasteryl stearate: white powder,[1]HNMR(CDCl3) δ 4.610 (m, 1H, H-3), 5.3685 (m, 1H, H-6), 0.696 (s, 3H, H-29), 5.154 (t, 1H,H-20), 5.016 (s, 3H, H-21), 1.021 (t, 3H, H-24), Parts of stearic acid: δ 0.880 (t, 3H, H-18), These data are in agreement with those reported in literature.[30,31]

The 3 compounds (HS1, HS2, and HS3) isolated from the DCM fraction were identified as stigmasterol (Figure 1), stigmasteryl palmitate (Figure 2), and stigmasteryl stearate (Figure 3) based on the NMR characteristics.

Husk extract of *Zea mays* has been shown previously to exert antihyperglycemic and antihyperlipidemic effects in alloxan-induced diabetic rats[15]. However, its antiobesity effect on high fat diet-fed rats and *in vitro* activity against pancreastic lipase has not been investigated to validate its folkloric usage to treat dyslipidemia. Therefore, this work was designed to evaluate *in vivo* and *in vitro* antiobesity activities of husk extract and fractions of *Zea mays.*

The husk extract and fractions were found to cause significant lowering of serum triglyceride, cholesterol, LDL, and VLDL in the high fat diet-fed rats with the DCM fraction exerting the highest effect during the *in vivo* study. These findings corroborate earlier report of its hypolipidemic activity in alloxan-induced diabetic rats[15]. The isolated compounds from DCM fraction (stigmasterol and stigmasterylpalmitate) showed weak inhibitory activity against porcine pancreatic lipase *in vitro* with IC_{50} values of 18.55 ± 1.08 and 14.36 ± 0.71µg/mL respectively. Thus, suggesting the involvement of these compounds in the observed antihyperlipidemic effect of this fraction. Stigmasterol has been reported to be involved in hypolipidemic activity of many plants.[31,32]Previous studies involving isolation of stigmasterol from plant extract had reported weak inhibitory activity on porcine lipase,[31,32] corroborating the findings of this study. Inhibitors of the enzyme pancreatic lipase, an important enzyme in dietary triacylglycerol absorption, are likely to reduce the digestion of dietary triglycerides and to a large extent suppress the absorption of fatty acids,[33]thus, resulting in decreased serum lipid levels as was observed in this study. Orlistat, a potent inhibitor of pancreatic lipase, reduced body weight, plasma triglyceride and cholesterol in obese patients[34]following chronic consumption. In this study, the husk extract and fractions were found to have potentials to lower triglyceride and cholesterol levels in the obese rats, while the isolated compounds from the DCM fraction exhibited pancreatic lipase inhibitory activity. These findings suggest that the hypolipidemic activities of the husk extract and fractions could be due to their lipase inhibitory activity. These results also corroborate that reported on onion skin extract by Kim,[35] which possessed pancreatic lipase inhibitory activity and also lowered blood triglyceride and cholesterol in rats fed high fat diet. Orlistat consumption is associated with diarrhoea.[33] This side effect was not observed with the administration of the husk extract which has the same lipase inhibitory activity like Orlistat. Hence, the husk extract could serve as an alternative hypolipidemic agent that may be useful in the management of diabetic complications and cardiovascular problems.

Serum AST, ALT and ALP levels are globally used major markers of liver damage. HFD administration has been reported to alter liver function parameters such as liver enzymes (serum AST, ALT and ALP) as well as lipid metabolism (HDLC and TG) in obese animals.[36,37] Significant increases in the levels of liver enzymes; AST, ALT and ALP, following HFD were observed in the HFD-fed rats in this study, thus suggesting induction of hepatic dysfunction. However, husk extract and fractions administration prevented the elevation of these enzyme levels; AST, ALT and ALP, indicating liver protective potential of the husk extract/fractions against HFD-induced liver damage. This suggests the ameliorative effect of the husk extract and fractions in preventing the effect of HFD and suppressing the activity of lipase enzymes thereby protecting the liver from injury as was observed in the low levels of the liver marker enzymes. This finding agrees with earlier reports of hepatoprotective potentials of the husk extract and fractions against alloxan-, paracetamol and carbon tetrachloride-induced hepatotoxicity in rats.[12-14]

Orlistat and Sibutramine used in this study have been reported to cause increases in both AST and ALT levels in HFD-induced rats despite their antiobesity effect.[38,39]Similar effect was observed in this study as rats treated with Orlistat showed increased levels of ALT and AST corroborating earlier reports.

It was observed in this study that rats administered with the husk extract and fractions of *Zea mays* had reduced body weight gains.This finding is in line with previous reports on other medicinal plants like *Cassia mimosoides* (ethanol extract) and *Salacia reticulate* (water extract), with pancreatic lipase inhibitory potentials and body weight gain suppressive activity in animal model.[40,41]

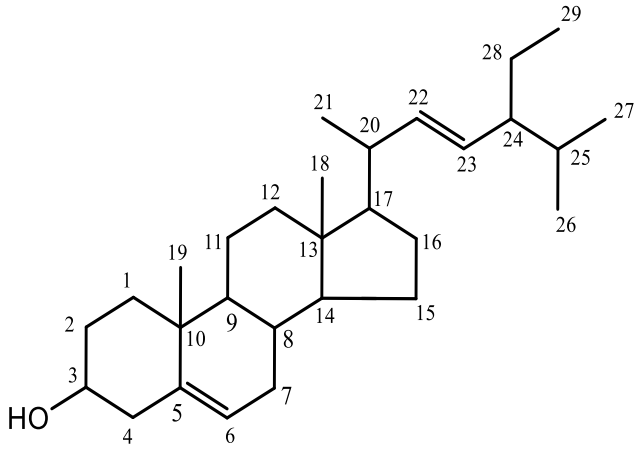

Figure 1: Chemical structure of Stigmasterol

Table 2: Effect of ethanol husk extract and fractions of *Zea mays* on Organ weights of HFD- induced obese rat

Treatment	Dose (mg/kg)	Weight of Organs (g)		
		Liver	Right Kidney	Left Kidney
Normal control	10 mg/ml	6.20 ± 0.10	0.95 ± 0.04	0.98 ± 0.06
Obese control		9.04 ± 0.36	1.34 ± 0.18	1.40 ± 0.22
Orlistat	5	7.53 ± 0.14 [a]	1.02 ± 0.24 [a]	1.08 ± 0.16 [a]
Crude extract	187	8.32 ± 0.64	1.11 ± 0.08	1.15 ± 0.14
	374	8.26 ± 0.55	1.16 ± 0.65	1.21 ± 0.15
	561	7.91 ± 0.48 [a]	1.13 ± 0.06	1.16 ± 0.08
n-Hexane fraction	374	7.86 ± 0.36	1.03 ± 0.08	1.10 ± 0.06
Dichloromethane fraction	374	6.89 ± 0.45 [a]	1.01 ± 0.18[a]	1.04 ± 0.05[a]
Ethyl acetate fraction	374	8.30 ± 0.78	1.14 ± 0.16	1.18 ± 0.08
Methanol fraction	374	8.27 ± 0.17	1.18 ± 13.17 [c]	1.20 ± 0.07

Data are expressed as Mean ± SEM, Significant at [a]$p < 0.05$, when compared to obese control. (n = 6).

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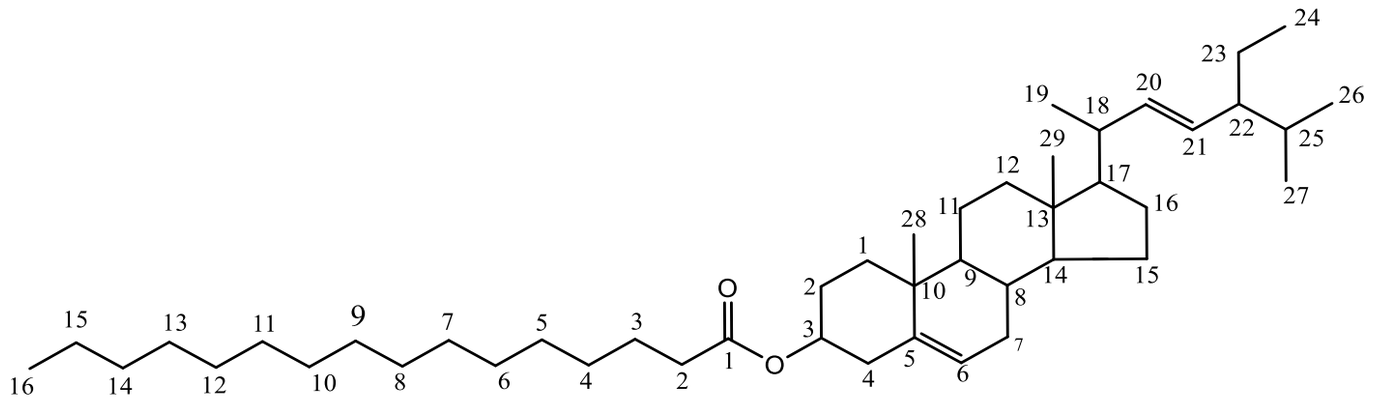

Figure 2: Chemical structure of Stigmasteryl palmitate

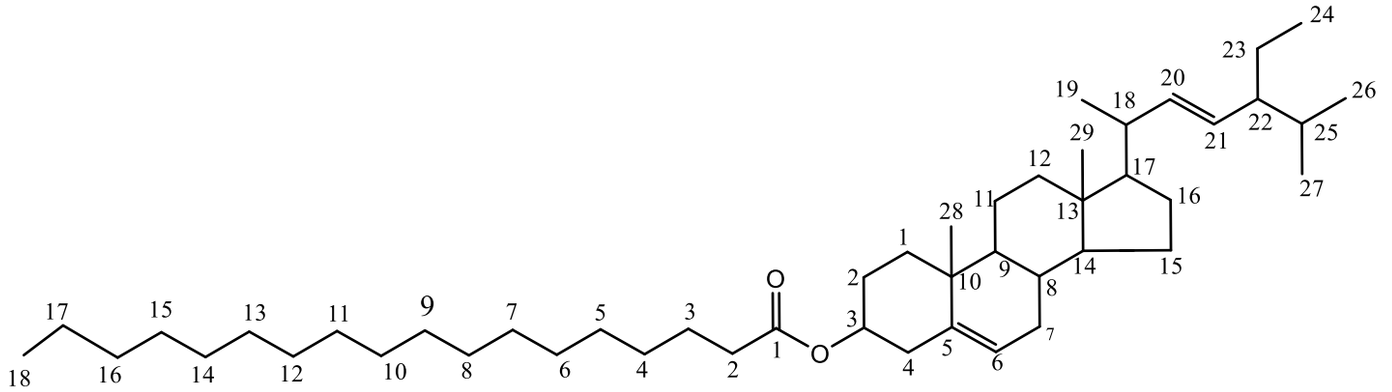

Figure 3: Chemical structure of Stigmasteryl stearate

Table 3: Effect of husk extract and fractions of *Zea mays* on lipid profile of HFD-induced obese rats

Treatment	Dose mg/kg	Total Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL (mg/dL)	Antherogenic Index (TC-HDL-C)/HDL-C	Blood Glucose Level
Normal Control	10 mL/kg	85.24 ± 3.16	60.56 ± 4.26	30.60 ± 2.31	36.05 ± 1.18	11.83 ± 3.01	1.78	73.2 ± 5.10
Obese Control	-	158.50 ± 21.65	171.23 ± 33.04	21.54 ± 2.16	110.92 ± 15.80	31.54 ± 5.68	6.35	161.2 ± 10.68
Orlistat	5	118.10 ± 12.64^{c}	109.46 ± 20.12^{b}	32.19 ± 9.83^{a}	49.24 ± 5.27^{a}	20.11 ± 3.48^{a}	2.69	91.40 ± 8.96^{c}
Crude extract	187	142.28 ± 20.15	151.38 ± 24.67^{a}	30.01 ± 12.85^{a}	75.44 ± 15.48^{c}	25.04 ± 8.42	3.74	128.62 ± 15.22^{c}
	374	119.37 ± 9.20^{c}	128.02 ± 5.33^{a}	31.34 ± 2.48^{a}	69.22 ± 8.70^{c}	22.49 ± 1.33^{a}	2.80	116.2 ± 21.88^{c}
	561	112.05 ± 11.19^{c}	120.68 ± 7.16^{a}	32.28 ± 5.36^{a}	55.82 ± 6.88^{c}	19.14 ± 4.35^{b}	2.47	99.26 ± 14.55^{c}
n-hexane fraction	374	111.42 ± 12.42^{c}	121.20 ± 15.28^{c}	30.92 ± 2.50^{c}	50.93 ± 8.35^{c}	20.22 ± 2.36^{c}	2.60	105.34 ± 12.66^{c}
Dichloromethane fraction	374	109.80 ± 0.67^{c}	116.22 ± 15.86^{b}	32.46 ± 4.19^{c}	45.86 ± 8.25^{c}	19.98 ± 4.15^{c}	2.38	98.87 ± 4.15^{c}
Ethyl acetate fraction	374	110.34 ± 15.06^{c}	120.02 ± 20.48^{c}	30.78 ± 5.96^{c}	48.21 ± 9.10^{c}	21.27 ± 3.14^{a}	2.58	109.3 ± 20.14^{c}
Methanol fraction	374	115.26 ± 12.44^{c}	126.83 ± 16.28^{b}	30.78 ± 6.35^{c}	51.36 ± 7.66^{c}	22.68 ± 5.45^{a}	2.74	102.18 ± 16.88^{c}

Data are expressed as MEAN ± SEM, Significant at $^{a}p<0.05$, $^{b}p< 0.01$, $^{c}p< 0.001$, when compared to obese control. (n = 6).

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Table 4: Effect of *Zea mays* husk extract and fractions on the liver function parameters of HFD-induced obese rats

Treatment	Dose (mg/kg)	Total Bilirubin (mg/dL)	ALT (IU/L)	ALP (IU/L)	AST (IU/L)
Normal control	10 (mg/ ml)	0.05 ± 0.01	33.67 ± 4.26	53.67 ± 2.03	81.00 ± 7.02
Obese Control	-	0.25 ± 0.01	55.0 ± 1.21	156.0 ± 0.57	162.81 ± 0.88
Orlistat	5	0.20 ± 0.02^{c}	50.33 ± 1.15^{c}	120.33 ± 3.71^{c}	130.33 ± 3.52
Crude extract	187	0.07 ± 0.01^{c}	31.33 ± 4.22^{c}	70.66 ± 1.76^{c}	98.33 ± 1.45
	374	0.06 ± 0.01^{c}	30.55 ± 1.49^{c}	73.25 ± 2.72^{c}	117.0 ± 11.06
	561	0.08 ± 0.01^{c}	28.24 ± 1.36^{c}	69.5 ± 3.92^{c}	108.25 ± 15.60^{b}
n- hexane Fraction	374	0.11 ± 0.01^{c}	30.66 ± 1.25^{c}	76.75 ± 3.25^{c}	132.75 ± 10.22^{c}
Dichloromethane fraction	374	0.08 ± 0.01^{c}	35.45 ± 1.45^{c}	87.25 ± 4.49^{c}	109.5 ± 17.24
Ethyl acetate fraction	374	0.09 ± 0.01^{c}	36.66 ± 2.25^{c}	98.33 ± 6.17^{c}	135.33 ± 14.19^{b}
Butanol Fraction	374	0.14 ± 0.01^{c}	34.0 ± 2.36^{c}	107.66 ± 9.95^{c}	142.33 ± 11.66^{b}

Data are expressed as Mean ± SEM, Significant at $^{a}p<0.05$, $^{b}p< 0.01$, $^{c}p< 0.001$, when compared to control. (n = 6)

Table 5: Effect of *Zea mays* husk extract and fractions on Liver antioxidative stress markers in HFD-induced obese rats

Parameters/ Treatment	Dose mg/kg	SOD (U/mg of protein)	CAT (U/mg of protein)	GPx (U/mg of protein)	GSH (µg/mg of protein)	MDA (µMol/mL)
Normal control		22.24 ± 0.24	55.52 ± 3.26	25.38 ± 0.66	0.35 ± 0.02	0.26 ± 0.04
Obese Control	-	10.28 ± 0.30^{b}	30.15 ± 0.16^{e}	12.23 ± 0.45^{d}	0.13 ± 0.01^{C}	0.56 ± 0.02^{C}
Orlistat	5	18.14 ± 0.22^{e}	45.34 ± 1.14^{f}	23.27 ± 0.48^{f}	0.33 ± 0.01^{f}	0.26 ± 0.06^{e}
Crude extract	187	12.38 ± 0.11	33.32 ± 0.99^{d}	17.99 ± 0.88^{d}	0.24 ± 0.01	0.38 ± 0.08^{d}
	374	15.17 ± 0.14	39.16 ± 1.12^{e}	19.73 ± 0.46^{d}	0.24 ± 0.01^{f}	0.29 ± 0.03^{e}
	561	16.16 ± 0.18^{e}	46.38 ± 2.19^{f}	21.39 ± 0.18^{e}	0.27 ± 0.02^{f}	0.21 ± 0.02^{f}
n -hexane fraction	374	19.02 ± 0.15^{e}	50.47 ± 1.92^{f}	22.11 ± 0.81^{f}	0.31 ± 0.01^{f}	0.21 ± 0.02^{f}
Dichloromethane fraction	374	21.14 ± 0.22^{f}	55.34 ± 1.14^{f}	23.27 ± 0.48^{f}	0.35 ± 0.01^{f}	0.22 ± 0.06^{e}
Ethyl acetate fraction	374	16.6 ± 3.72	47.98 ± 0.85^{f}	16.4 ± 2.65	0.27 ± 0.07^{f}	0.23 ± 0.09^{e}
n-butanol	374	18.2 ± 1.77^{d}	47.28 ± 0.41^{e}	19.8 ± 3.21^{d}	0.23 ± 0.65^{f}	0.25 ± 0.05^{e}

Data are expressed as mean ± SEM. significant at $^{d}p< 0.05$, $^{e}p< 0.01$, $^{f}p< 0.001$ when compared to obese control. n = 6.

Table 6: IC_{50}(*µ*g/ml) values of isolated compounds from DCM fraction of *Zea mays* husk on lipase activity

	Enzyme inhibitory activities
Compounds	**Lipase**
HS-1	18.55 ± 1.8
HS-2	14.36 ± 0.71
HS-3	NA
Positive control (Cetilistat)	7.17 ± 0.54

NA (not active)

Furthermore, atherosclerosis and coronary artery disease can be diagnosed from the atherogenic index[42,43]and increase in cholesterol level is a predisposal factor to the risk of fatty liver and atherosclerosis.[1] The results of this study indicated that these parameters were lowered in the husk extract/fractions-treated rats relative to those of the untreated HFD-fed group. Therefore, the findings of this study further suggest that husk extract/fraction has the potentials to lower the levels of the lipid parameters, and ameliorate cardiovascular disease, atherosclerosis and dyslipidemia in HFD-induced obesity. Obesity is also known to cause insulin resistance, which is prominent in type 2 diabetes. Previous works have reported high blood glucose and insulin levels in rats fed with HFD.[44,45] However, there are plant extracts with potentials to suppress the HFD-induced increase in blood glucose and insulin in obese animals.[46,47] Results of the present study show that HFD caused increases in serum glucose levels of the rats, while husk extract and fractions reduced these elevated levels of blood glucose significantly. Thus, confirming its antidiabetic activity as earlier reported.[15]

HFD-induced obesity exerts oxidative damage through lipid peroxidation, depletion of endogenous antioxidants and reduction of antioxidant enzymes activities, such as SOD, CAT and GPx.[48,49] In this study, HFD caused oxidative damage to rats as was obvious in the reduced levels of major endogenous antioxidant GSH, SOD, CAT and GPx activities. However, this oxidative stress activity was prevented by the administration of the husk extract and fractions as was seen in the increased levels of GSH, and SOD, CAT and GPx activities in the liver. These results corroborate the antioxidative stress activity of the husk extract and fractions earlier reported with other liver toxicants in previous studies.[12-14] This activity is likely due to the free radical scavenging activities of the phytochemical constituents of the husk extract. Plant extracts such as *Lavatera critica* leaf extract has also been reported to protect against obesity-induced oxidative damage in mice.[50]

Conclusion

The results of the study suggest that the husk extract and fractions have the potentials to prevent obesity in rats fed with high fat diet which is due to its hypolipidemic activity via lipase inhibitory activities of its phytochemical constituents.

Conflict of Interest

The authors declare no conflict of interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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