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Original Research Article

Phytochemical analysis and anti-obesity activities of *Vigna subterranea* (L.) Verdc. seed (Bambara nuts) fractions on alpha-amylase, pancreatic lipase and 3T3--L1 adipocytes

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Abstract

Purpose: *To evaluate the phytochemical constituents and anti-obesity activities of Vigna subterranea (L.) Verdc. seed (Bambara nut) fractions.*
Methods: *The hexane (HF), chloroform (CF), ethyl acetate (EF), and aqueous (AF) fractions from the ethanolic extract of Vigna subterranea were obtained using solvent-solvent partitioning with the respective solvents. Phytochemical analysis was performed using a liquid chromatograph mass spectrometer (LC–MS/MS-Q-TOF) on CF, EF, and AF in positive and negative ionization modes. The in vitro enzyme inhibitory activity of plant fractions was determined against α-amylase and pancreatic lipase, while the anti-adipogenic activity was assessed on 3T3-L1 preadipocytes.*
Results: *Phytochemical profiling revealed diverse metabolites belonging to the classes of fatty acids, flavonoids, alkaloids, saponins, peptides, and glycerophospholipids across the fractions. AF showed the most abundant compounds in both negative and positive modes, maintaining its superiority over other fractions as the most active against both α-amylase and pancreatic lipase, with inhibition greater than 50 % and IC_{50} of 250 ± 0.08 µg/mL and IC_{50} of 14.92 ± 0.15 µg/mL, respectively. The anti-adipogenic test exhibited a reduction in Oil Red O staining in AF-treated preadipocytes as the concentration increased, with significant reduction ($p < 0.05$) in intracellular lipid accumulation compared with the control treatment cells.*
Conclusion: *Fractions of V. subterranea contain diverse classes of bioactive compounds that may be responsible for inhibitory effects on digestive enzymes and adipogenesis suppression, suggesting they are potential sources of obesity therapy, particularly the AF. This finding provides the foundation for further studies.*

Keywords: *Adipogenesis, α-Amylase, Lipogenesis, LC–MS/MS-Q-TOF, 3T3-L1 preadipocytes, Pancreatic lipase, Vigna subterranea*

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INTRODUCTION

The World Health Organization (WHO) defines obesity as an abnormal increase in adipose tissue that results in a body mass index (BMI) ≥ 30 kg/m². It is regarded as a significant worldwide pandemic with both clinical implications and an economic burden on affected individuals [1]. Obesity is closely linked to an increased risk of noncommunicable diseases, including type 2 diabetes, heart disease, stroke, hypertension, certain cancers, and poor mental health. Additionally, obesity has been linked to increased susceptibility to communicable diseases, especially viral infections, as highlighted during the COVID-19 pandemic [2].

In 2022, about 2.5 billion adults (43%) aged 18 years and above were overweight, with over 890 million (16%) obese, making it 25% (14.0% men, 18.5 % women), higher than in 1990 (4.8% men and 8.8% women) globally [1]. By 2024, 35 million children under five were overweight. Among ages 5 – 19, more than 390 million were overweight, and 160 million were obese [3]. Several pharmacological weight management drugs are approved but often limited by side effects; some were withdrawn due to life-threatening issues like suicide [4]. Bioactive plant compounds have shown promise by acting via the same pharmacological mechanisms: suppression of appetite, modulation of adipogenesis, enhancement of energy expenditure, and inhibition of digestive enzymes [5,6].

Vigna subterranea (L.) Verdc. seed (Bambara groundnut) is a legume of Fabaceae, widely cultivated in sub-Saharan Africa and Asia to improve food security and support local markets. It is known for its high levels of compounds with nutritional and therapeutic benefits [7–9]. An ethnobotanical survey revealed that *Vigna subterranea*'s roots, leaves, and seeds are used in traditional medicine for obesity [10], abscesses, infected wounds, epilepsy, cataracts, venereal diseases, and inflammation [11].

Existing phytochemical investigations of *Vigna subterranea* (*V. subterranea*) have largely been limited to crude extracts analyzed by gas chromatograph mass spectrometer (GC–MS), providing only a partial understanding of its bioactive composition [8]. This study aims to identify phytochemicals in *V. subterranea* seed fractions using LC–MS/MS and evaluate their anti-obesity effects by inhibiting α-amylase and pancreatic lipase, and modulating adipogenesis in 3T3-L1 cells. These insights could clarify the plant's potential in inhibiting obesity-related enzymes and adipogenesis.

EXPERIMENTAL

Chemicals and reagents

Acarbose (CAS No.: 56180-94-0; Batch: 995GBC, Glentham Life Sciences, Leafield Way, UK), 4-nitrophenyl butyrate, 98 % (CAS No.: 2635-84-9; Sigma-Aldrich, St. Louis, MO, USA), lipase from porcine pancreas, Type II (EC 3.1.1.3; CAS No.: 9001-62-1; Sigma-Aldrich, St. Louis, MO, USA), and α-amylase from porcine pancreas, Type VI-B (EC 3.2.1.1; CAS No.: 9000-90-2; Sigma-Aldrich) were used in this study. Orlistat (tetrahydrolipstatin; marketed as Ecoslim™; CAS No.: 96829-58-2; Micro Labs Limited, Bangalore, India) was used as the reference drug.

Plant collection and identification

Vigna subterranea seeds were collected from local farmers in Auta-Balefi, Karu Local Government, located at approximately 8°56′48.8″ N latitude and 7°40′44.0″ E longitude of Nasarawa State, Nigeria, in May 2023. The seeds were identified and authenticated in the Department of Botany, National Institute of Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria, with NIPRD/H/7349 as the voucher specimen number for reference in the institution's herbarium.

Plant preparation, extraction and fractionation

Vigna subterranea seeds were washed to remove plant materials and insect debris, then air-dried under a shed for three weeks. Dried seeds were ground into powder using a local grinding mill and stored at room temperature at 20 – 25 °C in a zip-lock polystyrene bag before extraction procedures. Exactly 500 g of seed powder was macerated in 1,000 mL of absolute ethanol at room temperature, with intermittent shaking at 3–6 h intervals for 3 days. The mixture was first sieved using a muslin cloth (pore size 0.22 mm). The filtrate was again filtered using Whatman No. 1 filter paper. The resultant filtrate was concentrated to a slurry at 60 °C in a Biobase rotary evaporator (RE-501, Biobase Bioyu Co., Ltd.) fitted with a chiller and vacuum pump (SHZ-D(III), Biobase). Fractionation was carried out using solvent/solvent partitioning [12]. The concentrated crude extract was successively partitioned with hexane, chloroform, and ethyl acetate (3 × 300 mL each), leaving the aqueous

residue. Each fraction, hexane (HF), chloroform (CF), ethyl acetate (EF), and aqueous (AF), was concentrated and stored at 4 °C before analysis.

Phytochemical analysis

Liquid chromatography mass spectrometry analysis of V. subterranea fractions

Liquid chromatography mass spectrometry (LC–MS/MS) analysis was performed on CF, EF, and AF using an Agilent Eclipse XDB-C18 narrow-bore column (150 mm × 2.1 mm, 3.5 µm). The mobile phase was water with 0.1 % formic acid (A) and methanol with 0.1 % formic acid (B), with gradient elution from 8 % B to 90 % B over 40 min at 0.5 mL/min flow rate. Exactly 20 µL of the samples were injected, and electrospray ionization (ESI) in both positive and negative modes was used in the ionization procedure. The scan range was m/z 100 – 3200. Source parameters: capillary voltage 3500 V, fragmentor 125 V, skimmer 65 V, OCT 1 RF Vpp 750 V, drying gas 10 L/min at 300 °C. The data were analyzed using Agilent MassHunter Qualitative Analysis B.05.00.

Enzyme inhibitory assays

Alpha-amylase inhibitory assay

The enzyme-inhibitory effect of plant fractions at 2 mg/mL on α-amylase activity was evaluated by the modified starch-iodine method described by Xiao *et al* [13]. Reaction mixtures containing sodium phosphate buffer (pH 6.9, 0.02 M with 6 mM NaCl), starch solution (1 %), fractions (0 – 1000 µg/mL), and α-amylase (2 mg/mL) were incubated at 37 °C. After 15 min, the reaction was stopped with 1 M HCl, followed by iodine. Absorbance was read at 620 nm. Acarbose served as a positive control at 2 mg/mL. The IC_{50} value is the concentration of the inhibitor required to reduce enzyme activity by half (50 %). This was determined from a dose-response curve generated by plotting inhibitor concentrations against inhibition percentages, calculated using Eq 1.

Inhibition (%) = $(A_c\text{-}A_t/A_c)100$ …… (1)

Where A_t is the absorbance of the test sample and A_c is the absorbance of the control

Pancreatic lipase inhibitory assay

The inhibitory activity of the plant fractions at 2 mg/mL against pancreatic lipase was measured by adopting the p-nitrophenyl butyrate (p-NPB) method established by Kim *et al* [14] with some modifications. Reactions contained assay buffer (100 mM Tris-HCl, 5 mM $CaCl_2$, pH 7.0), enzyme (1 mg/mL), fractions/orlistat, and substrate (10 mM p-NPB). After incubation (37 °C, 15 min), absorbance was measured at 405 nm. The inhibition percentage of lipase activity by the plant fractions, positive and negative controls, was determined using Eq 2.

Lipase inhibition (%) = 100-{(B-b)/(A-a)}100 .. (2)

Where A = activity without inhibitor; a = negative control without inhibitor, B = activity with inhibitor, and b = negative control with inhibitor.

Cell culture and differentiation 3T3-L1 fibroblast (ATCC® CL-173™)

The differentiation of preadipocytes into adipocytes was performed using undifferentiated 3T3-L1 fibroblasts, 3T3-L1 (ATCC CL-173) cell lines. Preadipocytes were sustained in Preadipocyte Growth Medium (Ready-to-use; PromoCell, catalog No. C-27/410), a low-serum medium formulated for the expansion of undifferentiated preadipocytes. Cells were grown to 100 % confluency in either 24- or 6-well plates. Differentiation was initiated at Day 0 by replacing the growth medium with preadipocyte differentiation medium (PromoCell, catalog No. C-27/436), a defined, animal-component-free medium containing insulin, dexamethasone, 3-isobutyl-1-methylxanthine (IBMX), and the peroxisome proliferator-activated receptor gamma (PPARγ) agonist ciglitazone. After 72 hours (Day 3), the differentiation medium was changed to adipocyte nutrition medium (PromoCell, catalog No. C-27/438) to support the maturation and maintenance of the adipocytes. The nutrition medium was refreshed every 2 days thereafter. The differentiation process was complete by Day 10, as confirmed by the accumulation of lipid droplets.

Cell viability (MTT assay)

The effects of the fractions on the viability of 3T3-L1 preadipocyte cells were assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay described by Yang *et al* [15]. Briefly, cells were treated with plant fractions at concentrations of 0 (control), 31.25, 62.5, 125, and 250 µg/mL using serum-free Dulbecco's modified Eagle's medium (DMEM) for 48 hours. Following treatment, the cells were incubated with a 5 mg/mL MTT solution for 3 hours. The resulting formazan crystals were dissolved in 0.04 N hydrochloric acid (HCl) in

isopropanol, and absorbance was measured at 630 nm.

Oil Red 'O' Staining of intracellular triglycerides

Lipid accumulation in differentiated 3T3-L1 adipocytes was assessed using Oil Red 'O' staining. On day 10 post-differentiation, cells were gently washed twice with phosphate-buffered saline (PBS) and fixed using 10 % formalin for 30 minutes at room temperature. The formalin was removed after fixation, and the cells were washed twice with distilled water. The cells were then stained with a freshly prepared, filtered Oil Red 'O' working solution (0.5 % Oil Red 'O' Sigma-Aldrich in isopropanol diluted 3:2 with distilled water) for 1 hour at 18–25 °C. Following staining, the cells were washed thoroughly with water to elute unbound dye. For quantification, the retained Oil Red 'O' dye was eluted with 100 % isopropanol and was measured spectrophotometrically at 500 nm [16].

Statistical analysis

All the *in vitro* assay data were calculated in triplicate (n=3) and data expressed as Mean ± SEM after One-way Analysis of Variance (ANOVA) using Tukey's post hoc test at $p < 0.05$ on Statistical Package for the Social Sciences (SPSS) v23. Also, calculations and graphical visualizations were executed using GraphPad Prism version 5.0 (GraphPad Software, Inc., San Diego, CA, USA).

RESULTS

Phytochemical profiling of *Vigna subterranea* fractions

LC–MS/MS analysis of chloroform fraction (CF)

The LC–MS/MS analysis of the chloroform fraction (CF) in both ionization modes revealed flavonoids, alkaloids, saponins, and phenolic derivatives (Tables 1 & 2). In negative mode, twelve classes of seventeen compounds were identified, including organosulfur, alkaloid–derived, fatty acid derivatives, oxylipins, saponins, sesquiterpenoids, phenolic ketones, limonoids, steroidal glycosides, glycerophospholipids, alkyl sulfates, and sulfonated aromatics. Notable compounds include voglibose, 7,4'-dihydroxyflavone, salannin, and curcumenol. Sulfur-containing compounds such as butyl propyl disulfide, lauryl hydrogen sulfate, and N-undecylbenzenesulfonic acid were detected. The dominant chemical class is oxidized lipids (oxylipins), with five compounds including octadecenoic acid. In positive mode, nine compounds were identified, mainly fatty acids, along with glycerides, peptides, amino acids, a pyrone, and a terpenoid. Additionally, five endogenous fatty acid amides, including oleoyl ethanolamide, linoleoyl ethanolamide, and palmitoyl ethanolamide, were present.

LC–MS/MS analysis of ethyl acetate fraction (EF)

Table 3 lists compounds detected in negative ionization mode from the ethyl acetate fraction (EF) of *V. subterranea* seed, revealing 19 compounds across eight classes. The most abundant are sulfonated aromatics and fatty acids, with 7 and 6 compounds, respectively, including alkylbenzenesulfonic acids and both common and oxidized fatty acids such as oxylipins. Table 4 lists 13 compounds in positive mode, mostly fatty acids, including 2-hexyl-decanoic acid, 2-hydroxyhexadecanoic acid, and octadecadienoic acid. It also includes amino acid derivatives, peptides, a nucleobase (adenine), an amino alcohol, an alkaloid, an amide (decanamide), and the diterpenoid (-)-fusicoplagin A.

LC–MS/MS analysis of aqueous fraction (AF)

Table 5 details 72 compounds in the aqueous fraction (AF) under negative-ionization mode, including peptides, amino acid derivatives, glycerophospholipids, and alkaloids. Major classes were flavonoids (8), fatty acids (10), saponins (7), oxylipins (5), glycerophospholipids (5), and sulfonated aromatics (6). The positive-ionization analysis of AF identified 58 compounds, mainly amino acids and peptides like L-leucine, D-proline, Val–Met, and Lys–Gln–Phe. Lipids included five fatty acids, three oxylipins, monoglycerides, and sphingolipids (C16 sphinganine, dihydroceramide C2). Notably, five alkaloids, including loxtidine, were detected.

Enzyme inhibitory activity

Effect of fractions on α-amylase activities

The alpha-amylase inhibition properties of plant fractions (Figure 1 A) revealed the inhibitory potential of the various fractions against alpha-amylase to determine the role of the fractions in carbohydrate metabolism. Acarbose demonstrated the highest inhibitory activity at approximately 95 % at the concentration of 2 mg/mL, with an IC_{50} of 60 ± 0.08 µg/mL as the reference drug (positive control; Figure 1 B). This

established its efficacy as a standard alpha-amylase inhibitor, validating the assay conditions. The AF, EF, CF, and HF of *V. subterranea* (Figure 1 A) exhibited inhibitory activity of 62.33 %, 10.97 %, 25.36 %, and 18.22 %, respectively. Therefore, fractions with ≥ 50 % inhibition are considered active fractions. The most active fraction is AF of *V. subterranea* with an IC_{50} of 250 ± 0.08 µg/mL compared to acarbose (Figure 1 C), while EF, CF and HF were not active against alpha-amylase.

Effect of fractions on pancreatic lipase activities

The lipase inhibitory assay results (Figure 1 D) provide valuable insights into the fractions' potential anti-obesity properties through pancreatic lipase inhibition. The positive control, orlistat at 2 mg/mL, exhibited inhibition at approximately 60 % with an IC_{50} of 7.05 ± 0.19 µg/mL (Figure 1 E), serving as a standard for comparison. The AF, EF, CF and HF showed 69.49 %, 31.61 %, 66.53 %, and 31.34 % inhibitory activities, respectively, against pancreatic lipase.

Effects of fractions on 3T3-L1 preadipocytes

Cell viability assay

Adipocyte differentiation assays require that the test compounds not exert cytotoxic effects on precursor cells. Therefore, the cytotoxicity of *Vigna subterranea* seed fractions on 3T3-L1 undifferentiated adipocytes was first assessed using the MTT assay to determine safe concentrations for subsequent adipogenesis studies. Figure 2 revealed effects of aqueous (AF), hexane (HF), and ethyl acetate (EF) fractions of *V. subterranea* on 3T3-L1 preadipocytes. There was no significant reduction ($p > 0.05$) in cell viability at the concentrations of 31.25 µg/mL for AF and HF compared with the untreated control. Conversely, the concentrations of 62.5–250 µg/mL for AF and HF; and 31.25–250 µg/mL of CF and EF revealed significant decrease ($p < 0.05$) in cell viability compared with the untreated control.

Effects of AF on lipid content

Effect of AF on Relative Lipid Content in 3T3-L1 Preadipocytes

The effect of the aqueous fraction (AF) on lipid buildup was assessed by measuring the relative lipid content in 3T3-L1 cells, expressed as a percentage of the control (Figure 3). Treatment with AF at 31.25, 62.5, 125, and 250 µg/mL led to a significant decrease ($p < 0.05$) in lipid levels compared to untreated cells. The anti-adipogenic activity of AF was also visually confirmed through Oil Red 'O' staining, which marks lipid droplets as red patches in the cytoplasm (Figure 4). Cells treated with higher AF concentrations (31.25, 62.5, 125, and 250 µg/mL) exhibited progressively reduced lipid droplet size and number compared to the control cells. At 250 µg/mL, treated adipocytes lacked the typical spherical morphology of mature adipocytes, and lipid staining intensity was markedly lower than in controls, suggesting inhibition of differentiation.

DISCUSSION

This study is the first fraction-based liquid chromatography-mass spectrometry (LC–MS/MS) characterization of *V. subterranea* seeds, revealing more compounds than those reported in crude GC–MS analysis [7]. Quantitative LC–MS/MS profiling of chloroform, ethyl acetate, and aqueous fractions of *V. subterranea* seed was performed in both negative and positive ionization modes. A large portion of the fractions comprises lipid-related compounds, indicating a significant degree of lipophilicity. The AF contains the most abundant compounds, which were also observed in prior GC–MS profiling [8].

Fatty acids like octadecatrienoic acids, oxylipins (including 12-hydroxyjasmonic acid and 5,8,12-trihydroxy-9-octadecenoic acid), oleic and linoleic acids, and glycerophospholipids influence bioavailability and membrane interactions. They may disrupt membranes, inhibit enzymes like pancreatic lipase, promote fat oxidation, lower cholesterol, and boost thermogenesis. The plant's use for obesity is supported by terpenoids (curcumenol, mayolene), stimulants (theobromine), and surfactants (sulfonated aromatics), with theobromine and gingerol as phenolic ketone. These may raise basal metabolic rate (BMR), increase energy expenditure, and promote calorie burn through thermogenesis, with gingerol aiding digestion and gut health. Peptides such as Val–Trp–Glu and Ser–Trp–Val increase satiety, reduce overeating, and affect lipid metabolism [17]. Saponins and flavonoids support lipid metabolism and exhibit antioxidant and antiadipogenic effects. Compounds like Saponin E, gingerol, curcumenol, and octadecatrienoic acid are relevant to lipid metabolism, appetite, and oxidative stress in obesity.

Table 1: Some selected compounds in chloroform fraction of *Vigna subterranea* at negative polarity

Peaks No.	Compounds	Molecular Formula	Molecular Weight (g/mol)	Class
2	Butyl propyl disulfide	$C_7H_{16}S_2$	164.33	Organosulfur compound
3	Voglibose	$C_{10}H_{21}NO_7$	267.28	Alkaloid–derived drug
4	Nonic Acid	$C_9H_{16}O_4$	188.22	Fatty acid derivative
5	7,4'-Dihydroxyflavone	$C_{15}H_{10}O_4$	254.24	Flavonoid
6	11-hydroperoxy-12,13-epoxy-9-octadecenoic acid	$C_{18}H_{32}O_5$	328.45	Oxylipin
7	5,8,12-trihydroxy-9-octadecenoic acid	$C_{18}H_{34}O_5$	330.46	Oxylipin
15	9,10-Epoxy-18-hydroxystearate	$C_{18}H_{34}O_4$	314.46	Oxylipin
20	10,11-dihydroxy stearic acid	$C_{18}H_{36}O_4$	316.48	Oxylipin
23	12-oxo-10Z-octadecenoic acid	$C_{18}H_{32}O_3$	296.45	Oxylipin
8	Jujubasaponin IV	$C_{48}H_{78}O_{18}$	942.52	Saponin
9	Curcumenol	$C_{15}H_{22}O_2$	234.33	Sesquiterpenoid
11	Gingerol	$C_{17}H_{26}O_4$	294.39	Phenolic ketone
13	Salannin	$C_{34}H_{44}O_9$	596.73	Limonoid
14	Cucurbita-5,24-diene-3,7,23-triol 7-glucoside	$C_{36}H_{60}O_8$	620.43	Steroidal glycoside
18	Lauryl hydrogen sulfate	$C_{12}H_{26}O_4S$	266.4	Alkyl sulfate
21	N-Undecylbenzenesulfonic acid	$C_{17}H_{28}O_3S$	312.47	Sulfonated aromatic
24	2-Dodecylbenzenesulfonic acid	$C_{18}H_{30}O_3S$	326.5	Sulfonated aromatic

Table 2: Some selected compounds in chloroform fraction of *Vigna subterranea at* positive polarity

Peaks No.	Compounds	Molecular Formula	Molecular Weight (g/mol)	Class
23	Palmitoyl Ethanolamide	$C_{18}H_{37}NO_2$	299.49	Fatty acid amide
26	Oleoyl Ethanolamide	$C_{20}H_{39}NO_2$	325.53	Fatty acid amide
16	6,10-Octadecadiynoic acid	$C_{18}H_{28}O_2$	276.41	Fatty acid
18	2-Hydroxyhexadecanoic acid	$C_{16}H_{32}O_3$	272.42	Fatty acid
29	1-Monopalmitin	$C_{19}H_{38}O_4$	330.51	Glyceride
31	Lys Gln Phe	$C_{20}H_{31}N_5O_5$	421.5	Peptide
3	4-Hydroxy-6-methylpyran-2-one	$C_6H_6O_3$	126.11	Pyrone
8	Sphaerodiene	$C_{20}H_{36}$	276.5	Terpenoid
15	Hexadecatrienyl acetate	$C_{18}H_{30}O_2$	278.43	Fatty acid ester

Table 3: Some selected compounds in ethyl acetate fraction of *Vigna subterranea* (Negative Polarity)

Peaks No.	Compounds	Molecular Formula	Molecular Weight (g/mol)	Class
1	Theobromine	$C_7H_8N_4O_2$	180.16	Xanthine Alkaloid
2	Nonic Acid	$C_9H_{16}O_4$	188.22	Fatty acid
18	9Z,12Z,15E-octadecatrienoic acid	$C_{18}H_{30}O_2$	278.43	Fatty acid
23	6E,9E-octadecadienoic acid	$C_{18}H_{32}O_2$	280.45	Fatty acid
24	2-hexyl-decanoic acid	$C_{16}H_{32}O_2$	256.42	Fatty acid
25	16Z-octadecenoic acid	$C_{18}H_{34}O_2$	282.46	Fatty acid
3	11-hydroperoxy-12,13-epoxy-9-octadecenoic acid	$C_{18}H_{32}O_5$	328.45	Oxylipin
4	5,8,12-trihydroxy-9-octadecenoic acid	$C_{18}H_{34}O_5$	330.46	Oxylipin
8	9,10-Epoxy-18-hydroxystearate	$C_{18}H_{34}O_4$	314.46	Oxylipin
13	12-oxo-10Z-octadecenoic acid	$C_{18}H_{32}O_3$	296.45	Oxylipin
10	Lauryl hydrogen sulfate	$C_{12}H_{26}O_4S$	266.4	Sulfonated Aromatic
11	N-Undecylbenzenesulfonic acid	$C_{17}H_{28}O_3S$	312.47	Sulfonated Aromatic
14	2-Dodecylbenzenesulfonic acid	$C_{18}H_{30}O_3S$	326.5	Sulfonated Aromatic
5	Curcumenol	$C_{15}H_{22}O_2$	234.33	Sesquiterpenoid
6	Gingerol	$C_{17}H_{26}O_4$	294.39	Phenolic Ketone
22	Mayolene-18	$C_{36}H_{64}O_4$	560.48	Diterpenoid

Table 4: Some selected compounds in ethyl acetate fraction of *Vigna subterranea* (Positive Polarity)

No.	Compounds	Molecular Formula	Molecular Weight (g/mol)	Class
1	2-Amino-3-methyl-1-butanol	$C_5H_{13}NO$	103.16	Amino Alcohol
2	Adenine	$C_5H_5N_5$	135.13	Nucleobase
6	2-hexyl-decanoic acid	$C_{16}H_{32}O_2$	256.42	Fatty acid
9	2-Hydroxyhexadecanoic acid	$C_{16}H_{32}O_3$	272.42	Fatty acid
10	6E,9E-octadecadienoic acid	$C_{18}H_{32}O_2$	280.45	Fatty acid
8	D-Alanyl-(R)-lactate	$C_6H_{11}NO_4$	161.16	Amino Acid Derivative
12	Lys Gln Phe	$C_{20}H_{31}N_5O_5$	421.5	Peptide
4	decanamide	$C_{10}H_{21}NO$	171.28	Amide

Table 5: Some selected compounds in aqueous fraction of *Vigna subterranea* (Negative Polarity)

Peaks No.	Compounds	Molecular Formula	Molecular Weight (g/mol)	Class
1	Amaronol B	$C_{16}H_{14}O_8$	334.28	Flavonoid
19	3',4'-Dihydroxyflavone 4'-glucoside	$C_{21}H_{20}O_9$	416.38	Flavonoid Glycoside
20	Isovitexin	$C_{21}H_{20}O_{10}$	432.38	Flavonoid Glycoside
25	Demethyltexasin	$C_{15}H_{10}O_5$	270.24	Flavonoid
30	7,4'-Dihydroxyflavone	$C_{15}H_{10}O_4$	254.24	Flavonoid
34	Nodifloretin	$C_{16}H_{12}O_7$	316.27	Flavonoid
4	Dyphylline	$C_{10}H_{14}N_4O_4$	254.25	Xanthine Alkaloid
5	Theobromine	$C_7H_8N_4O_2$	180.16	Xanthine Alkaloid
3	5-Methylangelicin	$C_{12}H_8O_3$	200.19	Coumarin
24	Nonic Acid	$C_9H_{16}O_4$	188.22	Fatty acid
73	9Z,12Z,15E-octadecatrienoic acid	$C_{18}H_{30}O_2$	278.43	Fatty acid
80	6E,9E-octadecadienoic acid	$C_{18}H_{32}O_2$	280.45	Fatty acid
82	2-hexyl-decanoic acid	$C_{16}H_{32}O_2$	256.42	Fatty acid
83	16Z-octadecenoic acid	$C_{18}H_{34}O_2$	282.46	Fatty acid
17	12-hydroxyjasmonic acid	$C_{12}H_{18}O_4$	226.27	Oxylipin
18	(-)-12-hydroxy-9,10-dihydrojasmonic acid	$C_{12}H_{20}O_4$	228.29	Oxylipin
32	11-hydroperoxy-12,13-epoxy-9-octadecenoic acid	$C_{18}H_{32}O_5$	328.45	Oxylipin
33	5,8,12-trihydroxy-9-octadecenoic acid	$C_{18}H_{34}O_5$	330.46	Oxylipin
51	9,10-Epoxy-18-hydroxystearate	$C_{18}H_{34}O_4$	314.46	Oxylipin
65	12-oxo-10Z-octadecenoic acid	$C_{18}H_{32}O_3$	296.45	Oxylipin
10	Cynaroside A	$C_{21}H_{32}O_{10}$	444.48	Saponin
29	Calendasaponin C	$C_{54}H_{86}O_{25}$	1134.54	Saponin
31	Saponin E	$C_{42}H_{68}O_{14}$	796.99	Saponin
35	Lemmatoxin	$C_{48}H_{78}O_{18}$	942.51	Saponin
37	Lucyoside J	$C_{42}H_{66}O_{15}$	810.97	Saponin
38	Saponin E	$C_{42}H_{68}O_{14}$	796.99	Saponin
41	Dehydrosoyasaponin I	$C_{48}H_{76}O_{18}$	940.51	Saponin
45	Lucyoside Q	$C_{36}H_{58}O_9$	634.84	Saponin
27	p-Salicylic acid	$C_7H_6O_3$	138.12	Phenolic Acid
39	Curcumenol	$C_{15}H_{22}O_2$	234.33	Sesquiterpenoid
46	Gingerol	$C_{17}H_{26}O_4$	294.39	Phenolic Ketone
52	Salannin	$C_{34}H_{44}O_9$	596.71	Limonoid
79	Mayolene-18	$C_{36}H_{64}O_4$	560.9	Diterpenoid
72	27-nor-24S-methylcholestan-nonol	$C_{27}H_{48}O_9$	516.67	Steroid
61	Glycerophospho-N-Oleoyl Ethanolamine	$C_{23}H_{46}NO_7P$	479.59	Glycerophospholipid
57	Lauryl hydrogen sulfate	$C_{12}H_{26}O_4S$	266.4	Sulfonated Aromatic
62	N-Undecylbenzenesulfonic acid	$C_{17}H_{28}O_3S$	312.47	Sulfonated Aromatic
66	2-Dodecylbenzenesulfonic acid	$C_{18}H_{30}O_3S$	326.5	Sulfonated Aromatic

Table 6: Some selected compounds in aqueous fraction of *Vigna subterranea* (Positive Polarity)

No	Compound Name	Molecular Formula	Molecular Weight (g/mol)	Class
Amino Acid/Peptide (7 compounds)				
1	Val Met	$C_{10}H_{20}N_2O_3S$	248.34	Dipeptide
4	D-Proline	$C_5H_9NO_2$	115.13	Amino Acid
5	L-Leucine	$C_6H_{13}NO_2$	131.17	Amino Acid
46	Lys Gln Phe	$C_{20}H_{31}N_5O_5$	421.5	Tripeptide
47	Val Ser Arg	$C_{14}H_{28}N_6O_5$	360.42	Tripeptide
48	Lys Gln Phe	$C_{20}H_{31}N_5O_5$	421.5	Tripeptide
8	12-hydroxyjasmonic acid	$C_{12}H_{18}O_4$	226.27	Oxylipin
16	9,10-Epoxy-18-hydroxystearate	$C_{18}H_{34}O_4$	314.46	Oxylipin
17	1-Linoleoyl Glycerol	$C_{21}H_{38}O_4$	354.53	Monoglyceride
42	1-Monopalmitin	$C_{19}H_{38}O_4$	330.51	Monoglyceride
7	N-(3-Trifluoromethylphenyl) Piperazine (TFMPP)	$C_{11}H_{13}F_3N_2$	230.23	Alkaloid
38	Loxtidine	$C_{19}H_{29}N_5O_2$	359.48	Alkaloid
14	C16 Sphinganine	$C_{16}H_{35}NO_2$	273.46	Sphingolipid
19	Dihydroceramide C2	$C_{20}H_{41}NO_3$	343.55	Sphingolipid
21	Safingol (L-threo-sphinganine)	$C_{18}H_{39}NO_2$	301.51	Sphingolipid
12	8,2'-Dihydroxyflavone	$C_{15}H_{10}O_4$	254.24	Flavonoid
6	4-Hydroxy-6-methylpyran-2-one	$C_6H_6O_3$	126.11	Pyrone
10	Podolactone B	$C_{19}H_{22}O_9$	394.38	Terpenoid
20	Kolanone	$C_{33}H_{42}O_4$	502.69	Terpenoid
26	Eudesobovatol A	$C_{33}H_{44}O_4$	504.7	Terpenoid

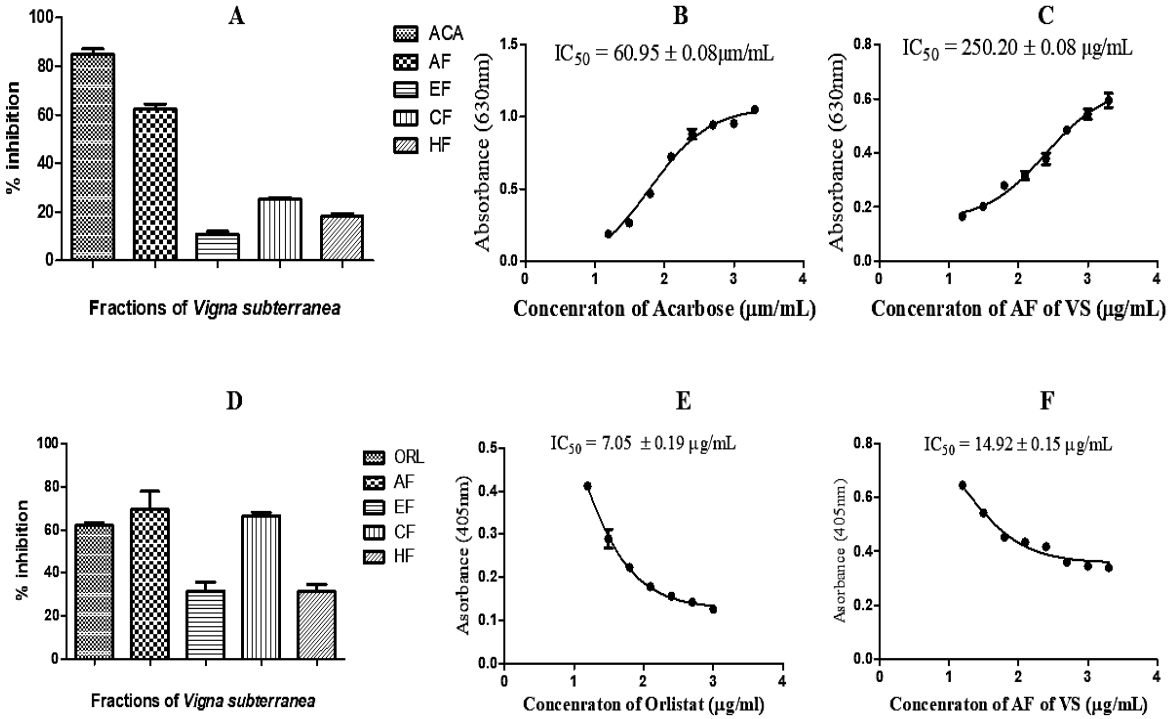


Figure 1: Effect of the fractions on α-amylase and pancreatic lipase activity compared with acarbose and orlistat (positive controls). (A) Alpha-amylase inhibition of *Vigna subterranea* fractions, (B) ACA IC_{50} against amylase, (C) AF IC_{50} against amylase, (D) Pancreatic lipase inhibition of VS fractions, (E) ORL IC_{50} against pancreatic lipase, (F) AF IC_{50} against lipase (F), ACA (Acarbose), ORL (Orlistat), AF (Aqueous fraction), EF (ethyl acetate), CF (Chloroform fraction), HF (Hexane fraction)

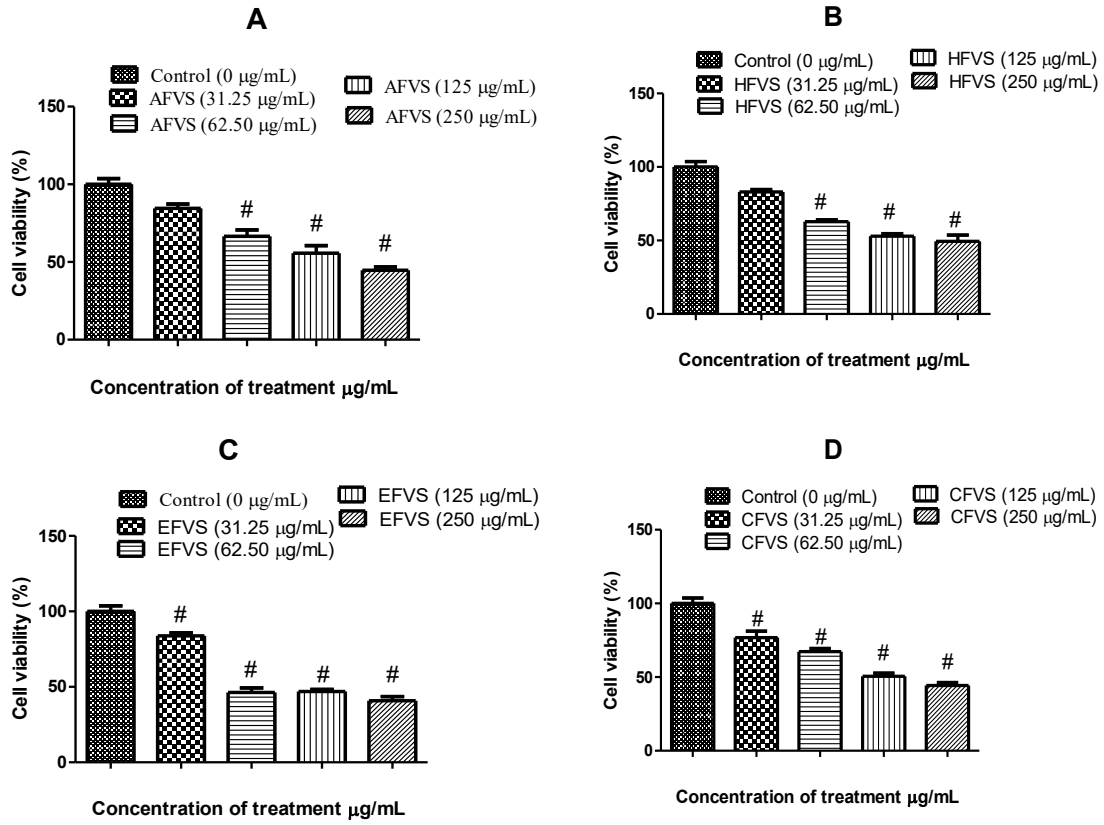


Figure 2: Cell viability assay (MTT) of 3T3-L1 adipocytes after treatment with fractions of VS compared to control. A(AF), B (HF), C (EF), D (CF). AF (Aqueous fraction), EF (ethyl acetate), CF (Chloroform fraction), HF (Hexane fraction), VS (*Vigna subterranea*). Control is at 0 µg/mL of plant fractions: n = 3

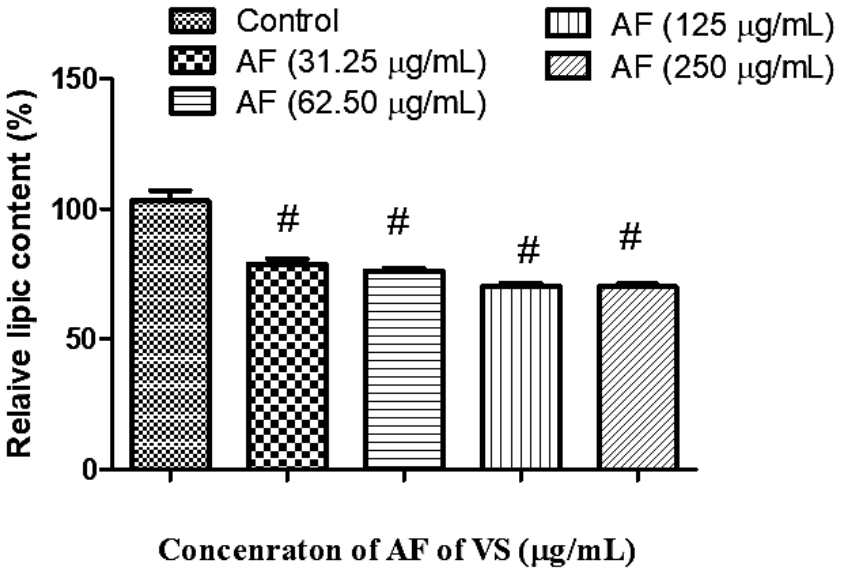


Figure 3: Effect of the aqueous fraction of *Vigna subterranea* on relative lipid content in differentiated 3T3--L1 adipocytes

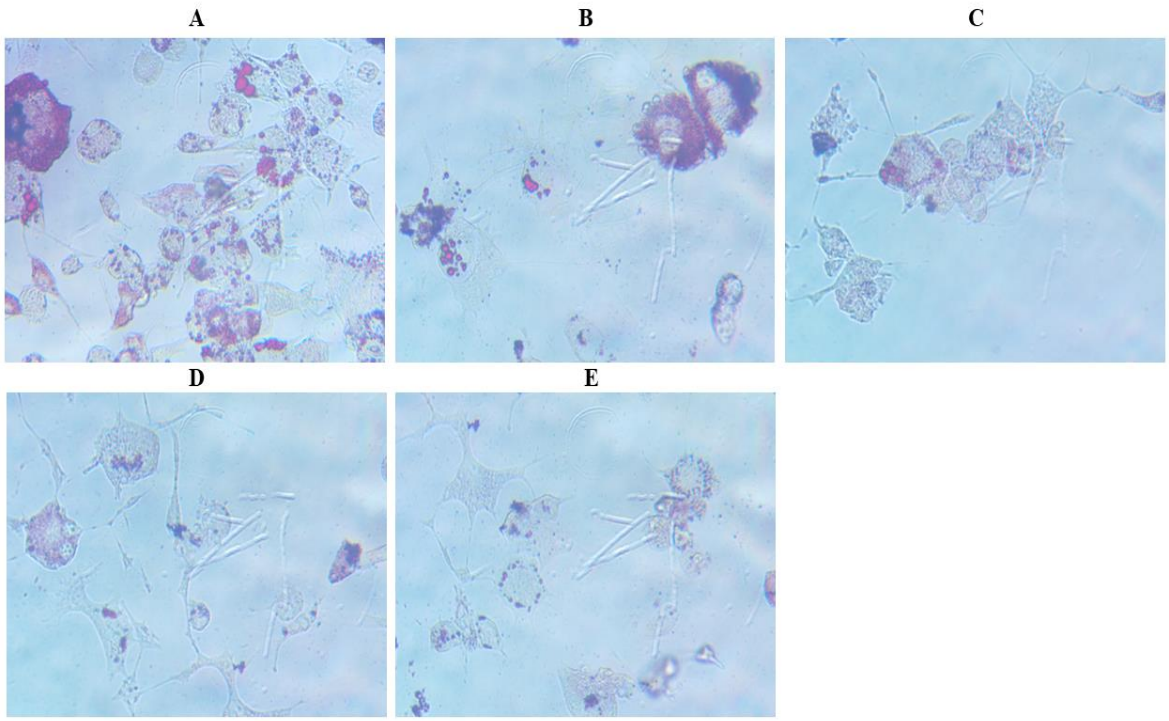


Figure 4: Oil Red 'O' staining of differentiated 3T3-L1 adipocytes treated with various fractions showing lipid accumulation reduction. A Control (DMSO); concentrations of aqueous fraction B (31.25 µg/mL), C (62.50 µg/mL), D (125 µg/mL), E (250 µg/mL)

Sulfur metabolites have obesity-related health benefits, supporting the plant's traditional use as an anti-obesity agent [18]. The AF showed strong inhibitory activity against alpha-amylase, with an IC_{50} of 250 ± 0.08 µg/mL. The inhibition of alpha-amylase prevents the breakdown of starch into glucose and oligosaccharides, which lowers total caloric intake and prevents sharp spikes in blood sugar and insulin, therefore preventing obesity [19]. . Thus, inhibition of alpha-amylase activity may be a strategic mechanism for achieving desirable weight loss. The fractions of *V. subterranea* showed promising amylase inhibition, with AF showing the highest potency among the fractions, suggesting that hydrophilic compounds are effective enzyme inhibitors. The AF of *V. subterranea* also showed inhibitory activity against pancreatic lipase, with an IC_{50} of 14.92 ± 0.15 µg/mL. Pancreatic lipase plays a crucial role in fat digestion and absorption in the gastrointestinal tract. Therefore, inhibitors of pancreatic lipases are considered anti-obesity agents. The AF also demonstrated lipase inhibitory activity, with significant anti-obesity properties. The inhibitory activities of this fraction on digestive enzymes and suppression of lipogenesis provide the first demonstration in *V. subterranea* fractions. The findings provide insight into a synergistic mechanism in which AF of *V. subterranea* reduces nutrient absorption by inhibiting digestive enzymes and limiting fat storage in adipocytes.

The AF and HF exhibited relatively higher cell viability, maintaining more than 80 % of viable cells at 31.25 µg/mL compared with control cells, indicating minimal cytotoxicity. However, metabolic activity was reduced compared with control cells; values remained above the 80 % viability threshold, indicating that these fractions are generally non-toxic at lower doses. At higher concentrations (62.5 – 250 µg/mL), viability decreased significantly, suggesting possible cytotoxic effects on 3T3-L1 preadipocytes. Overall, the MTT assay showed that the aqueous fraction (AF) preserved the highest cell viability across the tested concentrations, making it suitable for further studies on adipogenesis inhibition. These results imply that *V. subterranea* fractions, especially AF, have promising safety profiles and potential for anti-obesity strategies by influencing adipocyte differentiation. The decrease in lipid content is also reflected in reduced lipid intensity in Oil Red O, suggesting that compounds in *V. subterranea*'s AF may inhibit lipogenesis and pointing to a possible anti-obesity effect. It is important to note that glycerol release was not measured in this study. However, the inhibition of adipogenesis in preadipocytes implies

interference with key molecular mechanisms of differentiation, including transcription factors responsible for adipogenesis and post-translational processes responsible for lipid synthesis (lipogenesis). Compounds identified in *V. subterranea* fractions may inhibit or regulate adipocyte differentiation and lipid synthesis, including PPARγ, CCAAT/enhancer-binding protein alpha (C/EBPα), sterol regulatory element-binding protein-1c (SREBP-1c), and fatty acid synthase (FAS) [20]. The AF fraction emerged as the most potent, perhaps due to its enriched bioactive constituents.

CONCLUSION

Vigna subterranea seed has been used in traditional medicine to treat diseases. LC–MS/MS metabolite profiling and bioassays provide the first detailed chemical basis for evaluating *V. subterranea* as an anti-obesity option. The aqueous fraction's inhibition of α-amylase and pancreatic lipase indicates anti-obesity effects by reducing fat accumulation. These results show that the aqueous fraction inhibits digestive enzymes and reduces fat cell formation and fat production. This dual action supports *V. subterranea* as a natural anti-obesity therapy, validating traditional claims and guiding future research to isolate specific compounds.

DECLARATIONS

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Conflict of Interest

No conflict of interest observed in this work.

Contribution of Authors

We declare that this work was made possible by the authors whose names are in this article, and all liabilities regarding to claims in the content of this article will be borne by the authors. Dr Gabriel O. Anyanwu and Ivo Emo Andrew conceived and drafted the research protocol. Ivo Emo Andrew carried out the laboratory experiments under the supervision of Dr Gabriel O. Anyanwu and Dr. Uju Dorathy Ejike. Ivo Emo Andrew and Dr Gabriel O. Anyanwu performed the statistical analysis. Ivo Emo Andrew drafted the manuscript with the kind assistance of Dr. Gabriel O. Anyanwu, Dr. Rabi Abdu, and Dr. Richard James Banya. Shinjawa Yunana and Ijeoma Claira Osuagwu provided support in animal care, sample collection, laboratory analysis, and manuscript review. All the authors contributed to writing, reading, and approving the manuscript for publication.

Ethical approval

All ethical procedures were approved by Bingham University Teaching Hospital Health Research and Ethics Committee (BHUTH HREC), Jos, Plateau State, Nigeria, with no. NHREC/21/05/005/01398.

Availability of Data and Materials

Datasets used and/or analyzed during the current study shall be made available on request from the corresponding author.

REFERENCES

1. *WHO. 9 June. 2021. World Health Organization Obesity and Overweight. WHO Newsroom Fact Sheets: 2021.*
2. *Bansal V, Singh R, Bhurwal A, Rathore S, Kashyap R. 117: Obesity Is a Risk Factor for Increased COVID-19 Severity: A Systemic Review and Meta-Regression. Critical Care Medicine. 2021; 49(1). doi:10.1097/01.ccm.0000726356.00193.e7*
3. *Malnutrition in Children - UNICEF DATA [Internet]. [cited 2025 Sep 13]. Available from: https://data.unicef.org/topic/nutrition/malnutrition/?utm_source=chatgpt.com*
4. *Bays HE, Fitch A, Christensen S, Burridge K, Tondt J. Anti-Obesity Medications and Investigational Agents: An Obesity Medicine Association (OMA) Clinical Practice Statement (CPS) 2022. Obesity Pillars. 2022 Jun 1; 2:100018. doi:10.1016/j.obpill.2022.100018*
5. *Benbaibeche H, Boumehira AZ, Khan NA. Natural bioactive compounds and their mechanisms of action in the management of obesity: a narrative review. Front Nutr. 2025 Jun 26; 12:1614947. doi:10.3389/fnut.2025.1614947 PubMed PMID: 40642166; PubMed Central PMCID: PMC12243113.*
6. *Shaik Mohamed Sayed UF, Moshawih S, Goh HP, Kifli N, Gupta G, Singh SK, Chellappan DK, Dua K, Hermansyah A, Ser HL, Ming LC, Goh BH. Natural products as novel anti-obesity agents: insights into mechanisms of action and potential for therapeutic management. Front Pharmacol. 2023 Jun 20; 14:1182937. doi:10.3389/fphar.2023.1182937 PubMed PMID: 37408757; PubMed Central PMCID: PMC10318930.*
7. *Abdulmumin T, Yunusa A, Alhassan A, Muhammad I, Dalhatu M, Amina L, Bichi S, Sarki S, Sadiya A, Mikail*

T. Amino Acid Evaluation and GC-MS Analysis of Bambaranut (Vigna subterranea (L) Verdc.) †. Vol. 22. 2020 Mar 1; 22:78–86.

8. Adebiyi JA, Njobeh PB, Adebo OA, Kayitesi E. Metabolite profile of Bambara groundnut (Vigna subterranea) and dawadawa (an African fermented condiment) investigation using gas chromatography high resolution time-of-flight mass spectrometry (GC-HRTOF-MS). Heliyon. 2021 Apr 1;7(4):e06666. doi:10.1016/j.heliyon.2021.e06666
9. Nameni RO, Todem D, Woumbo CY, Goufani RBA, Tekou FA, Zokou R, Kuate D, Tamokou JDD. Nutritional, Antioxidant, and Functional Properties of Cameroonian Cowpea and Bambara Groundnut and Their Culinary Forms. Cureus. 2024 Nov 26;16(11):e74543. doi:10.7759/cureus.74543 PubMed PMID: 39735031.
10. Anyanwu GO, Anzaku D, Bulus YJ, Girgi JN, Donwell CC, Ihuma JO, Onyeneke EC, Bermano G, Steenkamp V. An Ethnobotanical Survey and Pharmacological and Toxicity Review of Medicinal Plants Used in the Management of Obesity in the North Central Zone of Nigeria. J Obes. 2025; 2025:5568216. doi:10.1155/jobe/5568216 PubMed PMID: 40026359; PubMed Central PMCID: PMC11870763.
11. Vigna subterranea [Internet]. [cited 2026 Oct 11]. Available from: https://prota.prota4u.org/protav8.asp?g=pe&p=Vigna+subterranea
12. Hostettmann K, Marston A, Hostettmann M. Separation Strategy and Combination of Methods. In: Hostettmann K, Marston A, Hostettmann M, editors. Preparative Chromatography Techniques: Applications in Natural Product Isolation [Internet]. Berlin, Heidelberg: Springer; 1998 [cited 2025 Apr 23]. p. 230–40. Available from: https://doi.org/10.1007/978-3-662-03631-0_10 doi:10.1007/978-3-662-03631-0_10
13. Xiao Z, Storms R, Tsang A. A quantitative starch–iodine method for measuring alpha-amylase and glucoamylase activities. Analytical Biochemistry. 2006 Apr 1;351(1):146–8. doi:10.1016/j.ab.2006.01.036
14. Kim YS, Lee YM, Kim H, Kim J, Jang DS, Kim JH, Kim JS. Anti-obesity effect of Morus bombycis root extract: Anti-lipase activity and lipolytic effect. Journal of Ethnopharmacology. 2010 Aug 9;130(3):621–4. doi:10.1016/j.jep.2010.05.053
15. Yang Z, Tu Y, Xia H, Jie G, Chen X, He P. Suppression of free-radicals and protection against H2O2-induced oxidative damage in HPF-1 cell by oxidized phenolic compounds present in black tea. Food Chemistry. 2007 Jan 1;105(4):1349–56. doi:10.1016/j.foodchem.2007.05.006
16. Roh C, Jung U. Screening of Crude Plant Extracts with Anti-Obesity Activity. International Journal of Molecular Sciences. 2012 Feb;13(2):1710–9. doi:10.3390/ijms13021710
17. Alhamoud Y, Wu J, Ahmad MI, Chen F, Feng F, Wang J. 6-Gingerol induces browning of white adipose tissue to improve obesity through PI3K/AKT-RPS6-mediated thermogenesis pathway. Food Frontiers. 2023 Sep;4(3):1285–97. doi:10.1002/fft2.251
18. Lee MS, Kim IH, Kim CT, Kim Y. Reduction of Body Weight by Dietary Garlic Is Associated with an Increase in Uncoupling Protein mRNA Expression and Activation of AMP-Activated Protein Kinase in Diet-Induced Obese Mice12. The Journal of Nutrition. 2011 Nov 1;141(11):1947–53. doi:10.3945/jn.111.146050 PubMed PMID: 21918057.
19. Kim DH, Park YH, Lee JS, Jeong HI, Lee KW, Kang TH. Anti-Obesity Effect of DKB-117 through the Inhibition of Pancreatic Lipase and α-Amylase Activity. Nutrients. 2020; 12(10):3053. https://doi.org/10.3390/nu12103053
20. Enayati A, Ghojoghnejad M, Roufogalis BD, Maollem SA, Sahebkar A. Impact of Phytochemicals on PPAR Receptors: Implications for Disease Treatments [Internet]. doi:10.1155/2022/4714914