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Chemical composition and *in vitro* antiobesity and *in vivo* anti-hyperlipidemic effects of Ceratotheca sesamoides, Jatropha tanjorensis, Mucuna flagellipes, Pterocarpus mildbraedii and Piper guineense

Gabriel O. Anyanwu[a,*], Dorathy Anzaku[a], Chinda C. Donwell[a], Usunomena Usunobun[b], Ademuyiwa J. Adegbegi[c], Patricia C. Ofoha[d], Khalid Rauf[e]

[a] Department of Biochemistry, Bingham University, Karu, Nasarawa State, Nigeria
[b] Department of Biochemistry, Faculty of Basic Medical Sciences, Edo University Iyamho, Edo State, Nigeria
[c] Department of Science Laboratory Technology, Rufus Giwa Polytechnic Owo, Ondo State, Nigeria
[d] Department of Biochemistry, University of Benin, Benin City, Edo State, Nigeria
[e] Department of Pharmacy, COMSATS University Islamabad, Abbottabad campus, Pakistan

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ABSTRACT

Ethnopharmacological relevance: Ceratotheca sesamoides, Jatropha tanjorensis, Mucuna flagellipes, Pterocarpus mildbraedii and Piper guineense are all medicinal plants found in West Africa and used traditionally for the treatment of various ailments such as; hypertension, arteriosclerosis, anemia, malaria and flatulence.

Aim of the study: The purpose of this study is to ascertain the in vitro anti-obesity and in vivo anti-hyperlipidemic activity of extracts and fractions of selected plants.

Materials and methods: The ethanolic extract of the aforementioned five plants were tested in vitro for their activity against porcine pancreatic lipase and alpha-amylase at a concentration of 1mg/ml. Thereafter, the IC_{50} values of the plants active against alpha-amylase were determined. The ethanolic extract of P. mildbraedii and M. flagellipes were fractionated using n-hexane, n-butanol, ethyl acetate and water, thus the four fractions each obtained were used for further in vitro and in vivo studies. Thirty-five male rats were divided into seven groups out of which groups 2–7 were made hyperlipidemic using poloxamer 407 (1.0 g/kg body weight) intraperitoneally. Groups 1, 2 and 3 were the normal, hyperlipidemic and standard drug control groups respectively, whereas groups 4–7 were the test groups.

Results: C. sesamoides, M. flagellipes and P. mildbraedii inhibited alpha-amylase activity, while M. flagellipes and P. mildbraedii had activity against pancreatic lipase. The n-hexane fraction of P. mildbraedii and M. flagellipes inhibited both lipase and amylase enzyme activities among the four fractions of each plant tested. The GCMS analysis of n-hexane fractions revealed the presence of two major compounds in each plant which were 9-octadecenoic acid and hexadecanoic acid in P. mildbraedii, and hexadecanoic acid and 9,12-octadecadienoic acid in M. flagellipes. The in vivo studies revealed that the n-hexane fraction of P. mildbraedii and M. flagellipes significantly decreased ($p < 0.05$) total triglycerides, total cholesterol, and low density lipoprotein cholesterols when compared to the hyperlipidemic control rats.

Conclusions: P. mildbraedii and M. flagellipes have the potency to act as antiobesity and anti-hyperlipidemic agents.

1. Introduction

Obesity is referred to as the excessive buildup of fat to the extent that it adversely affects health. It could also be defined as a Body Mass Index greater than or equal to 30 kg/m^2 (WHO, 2020). The worldwide prevalence of obesity has since been on the increase (Garvey et al., 2016), and as at 2016, about 13% of the world's adult population were obese (WHO, 2020). Obesity has now become one of the leading causes of death around the world (Jones et al., 2012), and has comorbidities such as insulin resistance, diabetes, hyperlipidemia, hypertension, heart attacks, stroke, osteoarthritis and

* Corresponding author.
E-mail address: gabrielanyanwu@binghamuni.edu.ng (G.O. Anyanwu).

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some cancers (Martorell et al., 2000; Rutter, 2011). The prevention of obesity is better than management/treatment which usually involves the use of drugs coupled with dieting and increased physical activity. Some antiobesity drugs include orlistat, naltrexone/bupropion, liraglutide, phentermine/topiramate and lorcaserin (Daneschvar et al., 2016).

Hyperlipidemia, which is often a comorbidity of obesity is usually characterized by high levels of total cholesterol, triglycerides, very low density lipoprotein, low density lipoprotein and decreased high density lipoprotein levels in the serum. Elevated total cholesterol levels of 240 mg/dl and above raises the risk of coronary heart diseases and stroke (Sheneni et al., 2018; CDC, 2020). In 2015–2016, above 12% of adults age 20 and older were found to have total cholesterol higher than 240 mg/dL in the United States (Carroll et al., 2017). Statins reduce total cholesterol and LDL-C by inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (Karr, 2017). Common statin drugs include atorvastatin, rosuvastatin, pitavastatin, lovastatin, simvastatin, fluvastatin and pravastatin.

Despite the existence of drugs for the treatment of obesity and hyperlipidemia, the search for bioactive extracts and compounds from medicinal plant sources is ongoing to increase the drug candidates, reduce adverse effect and cost, and increase availability. Medicinal plants contain substances that might be used for therapeutic purposes or as precursor for drug synthesis (WHO, 1998). Thus, in this study, crude extracts and fractions from selected medicinal plants were assayed as potential anti-obesity and anti-hyperlipidemic agents. These plants whose names have been verified on http://www.theplantlist.org were Ceratotheca sesamoides Endl. leaves, Jatropha tanjorensis J.L.Ellis & Saroja leaves, Mucuna flagellipes Hook.f seeds, Pterocarpus mildbraedii Harms leaves and Piper guineense Schumach. & Thonn seeds.

The local names of selected plants are C. sesamoides (common name-false sesame; yando & karkashi in Hausa language; Eku or E-kuku in Yoruba), J. tanjorensis (common names- Jatropha, catholic vegetables, 'Hospital too far', Iyana ipaja in Yoruba language); M. flagellipes (ukpo in Igbo), P. mildbraedii (Oha or Ora in Igbo language), and P. guineense (common names -Ashanti pepper, Benin pepper, Guinea pepper; Uziza in Igbo and Iyere in Yoruba) and these plants are commonly distributed in West Africa (Dalziel, 1955; Burkill, 1985). These plants have been reported to be safe for consumption and they possess one pharmacological activities such as antihypertensive, hepatoprotective, appetite suppressant, antimalarial, anti-diabetic, and anti-anaemic activities (Raji et al., 2003; Falodun et al., 2013; Egharevba et al., 2017; Hamzah et al., 2018; Pare et al., 2019).

2. Materials and methods

2.1. Plant material

Fresh parts of plants to be used were bought in April 2019 from Mararaba market at Karu local government area, Nasarawa state, Nigeria. Identification and authentication of plants was done by Dr. JO Ihuma, Department of Biological sciences, Bingham University, Nasarawa state, Nigeria. Voucher numbers were assigned to the plants which were deposited in the herbarium unit: Ceratotheca sesamoides leaves (B03601), Jatropha tanjorensis leaves (B4010), Mucuna flagellipes seeds (B03702), Pterocarpus mildbraedii leaves (B03704) and Piper guineense seeds (B03780).

2.2. Crude extract and fraction preparation

Fresh parts of the selected plants were cut into tiny pieces, dried at room temperature and grinded into powder. The pulverized plant materials were macerated in ethanol for 3 days at room temperature with shaking at intervals. Then, they were filtered with a muslin cloth first before using a filter paper and the filtrates were obtained. The filtrates were concentrated to slurry at different temperatures using a rotary evaporator. The plant crude extracts were dissolved in water and fractionated via the solvent/solvent partitioning method for the various organic solvents; hexane, n-butanol and ethyl acetate. Fractionation was carried out using the method as described by Hostettmann et al. (1991) with a few modifications.

2.3. Measurement of in vitro pancreatic lipase and alpha-amylase inhibitory activities

The inhibitory pancreatic lipase and α-amylase activities of the plant extracts/fractions were measured using the protocols described by Kim et al. (2009) and Xiao et al. (2006) respectively with some slight modifications which have been reported in Anyanwu et al. (2019).

2.4. Acute toxicity of fractions

The toxicity of the plant fractions was determined by the method described by OCDE (2002).

2.5. Experimental animals

Thirty-five male Wistar rats weighing between 150 and 175 g were acquired from National Veterinary Research Institute, Vom, Plateau State, Nigeria. The Wistar rats were housed in seven different cages in groups of five at the animal house in Bingham University, Karu, Nasarawa State, Nigeria. The rats were acclimatized at room temperature and 12:12 h light/dark cycle with free access to water and food for 2 weeks before onset of the study. Ethical approval for the studies was received from the ethical research committee of Bingham University, Karu, Nasarawa.

2.6. Induction of hyperlipidemia and treatment

Induction of hyperlipidemia was carried out according to the method as described by Wout et al. (1992) and Wasan et al. (2003) with modifications. Induction of hyperlipidemia in groups 2-7 using Poloxamer 407 (1 g/kg body weight) injection was done intraperitoneally and it was confirmed after three hours before the administration of plant fractions (150 and 300 mg/kg bw) and Rosuvastatin (75 mg/kg bw) was done orally using an oral gavage and normal saline as vehicle. The induction of hyperlipidemia was confirmed at 3 h post-P407 administration caused significant elevations in total cholesterol and triglyceride levels in rodents (Wout et al. 1992; Johnston and Palmer, 1993). Tail blood samples was collected from each animal immediately prior to poloxamer (1 g/kg body weight) injection and at 3 h to confirm induction, then following fraction and drug administration at 3, 24 and 48 h. Total cholesterol, triglycerides and high density lipoprotein levels were determined using mission cholesterol 3 in 1 measuring meter when a drop of blood was placed on the lipid panel. The test principle is based on enzymatic reaction to measure total cholesterol, triglycerides, HDL-cholesterol in samples when color is produced and detected by the mission cholesterol meter using reflectance photometry (Mission Cholesterol Test Device, ACON Laboratories, Inc., San Diego, USA).

2.7. Gas Chromatography Mass Spectrometry (GCMS) analysis of n-hexane fractions

The GCMS analysis of n-hexane fractions of Mucuna flagellipes seeds and Pterocarpus mildbraedii leaves was analyzed using Agilent GC 7890B, MS detector MSD 5977A (Agilent Technologies, USA) which is connected to a library software (MassHunter: NIST 14.L software) as described by Anyanwu et al. (2018).

Table 1
Effect of plants on α-amylase and lipase activities.

Plants	Extract/fraction	Pancreatic lipase IC_{50} (μg/mL)	α-amylase IC_{50} (μg/mL)
C. sesamoides	ethanol extract	-	658 ± 0.62
P. guineense	ethanol extract	-	-
J. tanjorensis	ethanol extract	-	-
P. mildbraedii	ethanol extract	19.87 ± 0.27	652.4 ± 0.94
	hexane fraction	9.74 ± 0.76	434.4 ± 0.50
	butanol fraction	-	-
	ethyl acetate fraction	-	-
	aqueous fraction	-	-
M. flagellipes	ethanol extract	15.88 ± 0.69	405 ± 2.28
	hexane fraction	10.67 ± 0.45	196 ± 0.67
	butanol fraction	8.25 ± 0.23	-
	ethyl acetate fraction	-	-
	aqueous fraction	-	-
Orlistat		0.089 ± 0.001	NA
Acarbose		NA	37.19 ± 0.71

Values are mean ± SEM, $n = 3$. NA- not applicable; (–) extracts/fractions did not inhibit 50% of enzymes at 1000 μg/mL.

Table 2
Effect of hexane fraction of plants on triglycerides levels (in mmol/L) of rats.

Treatment	0 h	3 h	24 h	48 h
Normal Control	0.617 ± 0.044^{a}	0.600 ± 0.029^{b}	0.637 ± 0.030^{d}	0.633 ± 0.038^{c}
Hyperlipidemic control	0.743 ± 0.097^{a}	3.037 ± 0.237^{a}	2.347 ± 0.094^{a}	2.033 ± 0.157^{a}
Rosuvastatin (75 mg/kg bwt)	0.907 ± 0.251^{a}	2.907 ± 0.179^{a}	1.897 ± 0.145^{b}	1.423 ± 0.061^{b}
P. mildbraedii (150 mg/kg bwt)	0.653 ± 0.076^{a}	3.282 ± 0.119^{a}	1.475 ± 0.020^{c}	1.398 ± 0.049^{b}
P. mildbraedii (300 mg/kg bwt)	0.629 ± 0.044^{a}	3.273 ± 0.172^{a}	1.597 ± 0.123^{bc}	1.318 ± 0.054^{b}
M. flagellipes (150 mg/kg)	0.647 ± 0.055^{a}	2.790 ± 0.224^{a}	1.977 ± 0.018^{b}	1.540 ± 0.021^{b}
M. flagellipes (300 mg/kg)	0.600 ± 0012^{a}	2.907 ± 0.030^{a}	1.587 ± 0.411^{b}	1.483 ± 0.371^{b}

Values are expressed as means ± SEM. Means sharing common letter(s) in the same column are not significantly different ($p < 0.05$).

Table 3
Effect of hexane fraction of plants on total cholesterol levels (in mmol/L) of rats.

Treatment	0 h	3 h	24 h	48 h
Normal Control	2.603 ± 0.009^{a}	2.600 ± 0.006^{a}	2.603 ± 0.009^{c}	2.620 ± 0.006^{c}
Hyperlipidemic control	2.600 ± 0.006^{a}	2.630 ± 0.006^{a}	4.613 ± 0.277^{ab}	6.610 ± 0.402^{a}
Rosuvastatin (75 mg/kg bw)	2.617 ± 0.012^{a}	2.630 ± 0.006^{a}	4.587 ± 0.102^{ab}	2.647 ± 0.052^{c}
P. mildbraedii (150 mg/kg bw)	2.630 ± 0.068^{a}	2.603 ± 0.048^{a}	5.705 ± 0.159^{a}	5.240 ± 0.140^{b}
P. mildbraedii (300 mg/kg bw)	2.620 ± 0.070^{a}	2.653 ± 0.080^{a}	4.190 ± 0.889^{b}	2.629 ± 0.115^{c}
M. flagellipes (150 mg/kg bw)	2.617 ± 0.008^{a}	2.617 ± 0.120^{a}	4.117 ± 0.880^{b}	2.640 ± 0.721^{c}
M. flagellipes (300 mg/kg bw)	2.617 ± 0.007^{a}	2.637 ± 0.009^{a}	4.433 ± 1.042^{ab}	2.710 ± 0.131^{c}

Values are expressed as means ± SEM. Means sharing common letter(s) in the same column are not significantly different ($p < 0.05$).

Table 4
Effect of hexane fraction of plants on high density lipoprotein levels (in mmol/L) of rats.

Treatment	0 h	3 h	24 h	48 h
Normal Control	0.397 ± 0.007^{a}	0.423 ± 0.009^{b}	0.397 ± 0.003^{c}	0.413 ± 0.012^{a}
Hyperlipidemic control	0.417 ± 0.015^{a}	1.457 ± 0.009^{a}	2.533 ± 0.019^{a}	0.460 ± 0.030^{a}
Rosuvastatin (75 mg/kg bwt)	0.407 ± 0.012^{a}	1.287 ± 0.530^{a}	2.327 ± 0.284^{a}	0.467 ± 0.050^{a}
P. mildbraedii (150 mg/kg bw)	0.386 ± 0.015^{a}	1.228 ± 0.124^{a}	2.591 ± 0.001^{a}	0.415 ± 0.037^{a}
P. mildbraedii (300 mg/kg bw)	0.380 ± 0.015^{a}	1.215 ± 0.182^{a}	1.857 ± 0.733^{b}	0.446 ± 0.037^{a}
M. flagellipes (150 mg/kg bw)	0.410 ± 0.015^{a}	1.057 ± 0.600^{a}	0.410 ± 0.006^{c}	0.403 ± 0.009^{a}
M. flagellipes (300 mg/kg bw)	0.400 ± 0.006^{a}	0.447 ± 0.120^{b}	0.430 ± 0.015^{c}	0.407 ± 0.009^{a}

Values are expressed as means ± SEM. Means sharing common letter(s) in the same column are not significantly different ($p < 0.05$).

2.8. Statistical analysis

Results from the in vivo studies were reported as mean ± Standard Error of Mean. Data analysis was carried out using one-way ANOVA using the SPSS version 16 with Tukey's multiple range test at $p < 0.05$ statistical level of significance. The IC_{50} values from the in vitro studies were calculated using Graphpad prism 5 software.

3. Results

3.1. In vitro pancreatic lipase and alpha-amylase inhibitory activities

The ethanolic extracts of M. flagellipes, and P. mildbraedii inhibited pancreatic lipase and alpha-amylase activities, but C. Sesamoides inhibited only amylase activity at a concentration of 1 mg/mL (Table 1). Also,

Table 5
Effect of hexane fraction of plants on CHOL/HDL ratio of rats.

Treatment	0 h	3 h	24 h	48 h
Normal Control	6.540 ± 0.150^{a}	6.147 ± 0.134^{a}	6.560 ± 0.055^{a}	6.347 ± 0.202^{b}
Hyperlipidemic control	6.253 ± 0.207^{a}	1.805 ± 0.119^{bc}	1.820 ± 0.114^{b}	14.370 ± 0.130^{a}
Rosuvastatin (75 mg/kg bw)	6.447 ± 0.204^{a}	2.044 ± 0.327^{bc}	1. 971 ± 0.265^{b}	5.668 ± 0.598^{b}
P. mildbraedii (150 mg/kg bw)	6.830 ± 0.354^{a}	2.120 ± 0.274^{bc}	2.202 ± 0.061^{b}	12.627 ± 0.061^{a}
P. mildbraedii (300 mg/kg bw)	6.928 ± 0.483^{a}	2.184 ± 0.355^{bc}	2.256 ± 0.395^{b}	5.895 ± 0.321^{b}
M. flagellipes (150 mg/kg bw)	6.220 ± 0.333^{a}	2.476 ± 0.353^{b}	10.07 ± 2.250^{a}	6.550 ± 0.754^{b}
M. flagellipes (300 mg/kg bw)	6.543 ± 0.081^{a}	5.899 ± 0.146^{a}	10.20 ±2.202^{a}	6.670 ± 0.372^{b}

Values are expressed as means ± SEM. Means sharing common letter(s) in the same column are not significantly different ($p < 0.05$).

Table 6
Effect of hexane fraction of plants on LDL levels (in mmol/L) of rats.

Treatment	0 h	3 h	24 h	48 h
Normal Control	1.927 ± 0.033^{a}	1.907 ± 0.007^{a}	1.920 ± 0.015^{a}	1.920 ± 0.006^{bc}
Hyperlipidemic control	1.850 ± 0.046^{a}	1.780 ± 0.096^{a}	2.017 ± 0.283^{a}	3.310 ± 0.376^{a}
Rosuvastatin (75 mg/kg bw)	1.800 ± 0.125^{a}	0.740 ± 0.301^{b}	1.397 ± 0.313^{b}	1.530 ± 0.104^{c}
P. mildbraedii (150 mg/kg bw)	1.946 ± 0.065^{a}	0.067 ± 0.195^{b}	2.444 ± 0168^{a}	2.189 ± 0.156^{b}
P. mildbraedii (300 mg/kg bw)	1.954 ± 0.067^{a}	0.100 ± 0.062^{b}	0.941 ± 0.948^{b}	1.584 ± 0.100^{bc}
M.flagellipes (150 mg/kg bw)	1.573 ± 0.050^{b}	1.707 ± 0.046^{a}	2.203 ± 0.875^{a}	2.317 ± 0.484^{b}
M. flagellipes (300 mg/kg bw)	1.947 ± 0.007^{a}	1.893 ± 0.007^{a}	2.283 ± 0.907^{a}	1.627 ± 0.227^{b}

Values are expressed as means ± SEM. Means sharing common letter(s) in the same column are not significantly different ($p < 0.05$).

Table 7
Compounds of n-hexane fraction (nHF) of P. mildbreadii.

PK	RT	Library/ID	Area (%)
1	24.43	Oxalic acid, allyl pentadecyl ester	0.33
2	28.89	Cetene	0.45
3	29.58	1-Trifluoroacetoxy-10-undecene	0.54
4	29.91	10-Undecen-1-ol	0.17
5	30.12	Pentadecanal-	0.27
6	30.62	Hexadecanoic acid, methyl ester	25.96
7	31.03	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	0.28
8	31.17	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	0.18
9	31.27	Hexadecanoic acid, ethyl ester	2.72
10	31.37	Oleic Acid	0.34
11	32.09	9,15-Octadecadienoic acid, methyl ester, (Z,Z)-	4.66
12	32.13	9-Octadecenoic acid, methyl ester, (E)-	35.73
13	32.24	Phytol	7.40
14	32.30	Methyl stearate	7.15
15	32.58	trans-13-Octadecenoic acid	2.62
16	32.69	Hexadecanoic acid, butyl ester	1.14
17	32.74	E-11-Hexadecenoic acid, ethyl ester	1.05
18	33.08	4-Hydroxyphenyl pyrrolidinyl thione	0.24
19	33.27	3-Isopropoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane	0.27
20	33.35	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	0.19
21	33.37	1H-isoindole-1,3(2H)-dione, 5-(ethylthio)-	0.18
22	33.61	Pyridine-3-carboxylic acid, 1,4-dihydro-5-cyano-2-hydroxy-4-(4-isopropylphenyl)-6-methyl-, ethyl ester	0.33
23	33.85	n-Propyl 11-octadecenoate	5.63
24	34.00	2-Ethylacridine	0.32
25	34.20	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester	0.21
26	35.26	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	0.32
27	35.30	Adamantane, 1-isothiocyanato-3-methyl-	1.07
28	36.58	Silicic acid, diethyl bis(trimethylsilyl) ester	0.24

both pancreatic lipase and alpha amylase activities were inhibited by n-hexane fractions of M. flagellipes, and P. mildbraedii, and only butanol fraction of P. mildbraedii inhibited pancreatic lipase activities.

3.2. Acute toxicity of fractions

The hexane fractions of M. flagillipes seeds and P. mildbraedii leaves caused zero death of rats after receiving 0– 2000 mg/kg bw single oral dose. Based on observations no adverse effect of these fractions were observed between 0-14 days in this study.

3.3. Effects of P. mildbraedii and M. flagillipes on lipid levels in treated rats

The induction of hyperlipidemia by Poloxamer 407 resulted in significant increase ($p < 0.05$) in TG levels of the rats in the induced groups when compared to normal control after 3 h (Table 2). At 24hrs and 48hrs there was significant decrease ($p < 0.05$) in TG levels of the rats treated with either Rosuvastatin, M. flagillipes and P. mildbraedii fractions when compared to the hyperlipidemic control. For Total cholesterol, at 48 h there was significant decrease ($p < 0.05$) in TC levels of the rats for all the treated groups compared to hyperlipidemic control,

Table 8
Compounds of n-hexane fraction (nHF) of M. flagellipes.

PK	RT	Library/ID	Area (%)
1	19.29	Oxalic acid, allyl hexadecyl ester	0.22
2	24.44	Bromoacetic acid, tetradecyl ester	0.13
3	28.44	13-Oxabicyclo[10.1.0]tridecane	0.38
4	28.56	cis-11-Hexadecenal	0.28
5	28.69	9-Oxabicyclo[6.1.0]nonane, cis-	0.46
6	28.77	9,12-Octadecadienoic acid (Z,Z)-	0.42
7	28.90	1-Eicosene	1.23
8	29.00	9,17-Octadecadienal, (Z)-	0.35
9	29.13	9,12-Octadecadienoic acid (Z,Z)-	0.84
10	29.20	11-Dodecen-1-ol trifluoroacetate	0.83
11	29.27	9,12-Octadecadienal	0.19
12	29.33	2-Methyl-Z,Z-3,13-octadecadienol	0.36
13	29.45	9,12-Octadecadienoic acid (Z,Z)-	1.11
14	29.55	9,17-Octadecadienal, (Z)-	1.14
15	29.65	Linoelaidic acid	0.98
16	29.72	9,12-Octadecadienoic acid (Z,Z)-	0.46
17	29.84	Linoelaidic acid	1.08
18	29.89	9,12-Octadecadienoic acid (Z,Z)-	0.79
19	30.04	9,17-Octadecadienal, (Z)-	0.81
20	30.13	9,12-Octadecadienoic acid (Z,Z)-	0.73
21	30.16	Oleic Acid	0.31
22	30.45	Linoelaidic acid	3.98
23	30.56	9-Oxabicyclo[6.1.0]nonane, cis-	2.02
24	30.62	Hexadecanoic acid, methyl ester	7.15
25	30.79	9,12-Octadecadienoic acid (Z,Z)-	1.54
26	30.86	9,17-Octadecadienal, (Z)-	2.31
27	30.95	9,12-Octadecadienoic acid (Z,Z)-	0.94
28	31.08	n-Hexadecanoic acid	13.51
29	31.27	Hexadecanoic acid, ethyl ester	7.49
30	31.82	Linoelaidic acid	3.60
31	31.97	9,12-Octadecadienoic acid (Z,Z)-	3.49
32	32.08	9,12-Octadecadienoic acid (Z,Z)-	6.16
33	32.12	cis-Vaccenic acid	6.76
34	32.30	Methyl stearate	1.37
35	32.46	9,12-Octadecadienoic acid (Z,Z)-	13.33
36	32.54	Linoleic acid ethyl ester	3.61
37	32.58	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	6.34
38	32.74	Octadecanoic acid, ethyl ester	2.23
39	33.07	2-Propen-1-amine, N-2-propenyl-	0.36
40	33.83	3H-indole, 2-methyl-3-phenyl-	0.11
41	34.24	1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-	0.31
42	35.28	2-(Heptyloxycarbonyl)benzoic acid	0.31

and a significant increase ($p < 0.05$) in hyperlipidemic control rats compared to normal control rats (Table 3).

The HDL-c levels in the treated rats had no significant difference ($p < 0.05$) when compared to normal control and hyperlipidemic control after 48 h (Table 4). The CHOL/HDL level of the rats treated with P. mildbraedii (300 mg/kg) and M. flagellipes (150 and 300 mg/kg) were significantly decreased ($p < 0.05$) when compared to hyperlipidemic control but not the normal control at 48 h (Table 5). Again, at 48 h, the groups treated with n-hexane fractions of P. mildbraedii and M. flagellipes had significantly decreased LDL-c levels when compared to the hyperlipidemic control (Table 6).

3.4. GCMS analysis of hexane fraction (HF) of P. mildbraedii and M. flagellipes

The n-hexane fraction of P. mildbraedii revealed the presence of twenty-eight (28) peaks depicting 24 compounds (Table 7), of which there were 5 major compounds: 9-octadecenoic acid, methyl ester, hexadecanoic acid, methyl ester, phytol, methyl stearate and n-propyl 11-octadecenoate. The n-hexane fraction of M. flagellipes revealed the presence of forty two (42) peaks depicting 19 compounds (Table 7), of which there were 6 major compounds: hexadecanoic acid, 9,12-octadecadienoic acid, cis-vaccenic acid, 9-octadecenoic acid (z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester, linoelaidic acid and linoleic acid ethyl ester (Table 8).

4. Discussion

4.1. Effect of plants on alpha-amylase activity

The inhibition of alpha-amylase activity in vitro by C. sesamoides, M. flagellipes and Pterocarpus mildbraedii supports their use as potential anti-obesity agents. The reduction of carbohydrate digestion and absorption via inhibition of α-amylase enzyme which catalyzes the hydrolysis of α-(1,4)-D-glycosidic linkages of starch and other glucose polymers is an important mechanism for antiobesity candidates (Mahmood, 2016). Studies carried out with natural products such as Clematis vitalba has shown and supported the use of plants in weight management (Marrelli et al., 2013), with the same mechanism of action by inhibiting the alpha-amylase enzyme and thus preventing the digestion of carbohydrates and promoting weight reduction. The bioactive ingredients responsible for the anti-amylase activities of P. mildbraedii and M. flagellipes are concentrated in their n-hexane fractions. Notwithstanding, the fractions that were not active could probably act through another mechanisms of action other than through inhibition of the alpha-amylase and/or lipase enzyme.

4.2. Inhibitory effect of selected plants against pancreatic lipase activity

Among the plants screened in vitro for their activity against pancreatic lipase, only M. flagellipes and P. mildbraedii inhibited pancreatic lipase validating their local use as weight reduction agents and potential

anti-obesity agents. Several studies have shown that inhibition of pancreatic lipase is one of the most studied mode of action for determining the efficacy of natural products as anti-obesity agents (Anyanwu et al., 2019). Generally, evidences abound to support the increasing consumption of medicinal plants as an effective strategy for obesity control and weight management (Karri et al., 2019). The ability of these plants to inhibit the action of pancreatic lipase in vitro indicates that it could decrease the absorption of dietary fat by the intestine since it can only be directly absorbed after being acted upon by pancreatic lipase, thus decreasing the amount of fat that can be taken up for storage in the adipocytes, reducing the risk of obesity and improving weight reduction.

The n-hexane fraction of P. mildbraedii, and the n-hexane and butanol fraction of M. flagellipes were the only fractions active against the lipase enzyme implying that the compound(s) responsible for the inhibition of pancreatic lipase in the plants are probably concentrated in these fractions. These natural compounds could be fatty acids, polyphenols, flavonoids, and saponins amongst many others which have been found in several natural products used in weight management that act via a mechanism where they inhibit pancreatic lipase which is the key enzyme in dietary fat absorption and hydrolysis (Birari et al., 2007).

4.3. Effect of the n-hexane fraction of P. mildbraedii and Mucuna flagellipes on hyperlipidemic rats

In accordance with previous studies, increase in lipid levels were observed after successful induction of hyperlipidemia using Poloxamer 407 at a concentration of 1 g/kg (Wout et al., 1992; Wasan et al., 2003). The n-hexane fractions of 150 and 300 mg/kg of P. mildbraedii and M. flagellipes decreased the TC, TG and LDL levels of the rats which was elevated by the intraperitoneal injection of Poloxamer 407. Apparently, P-407 stimulates the activity of the rate limiting enzyme 3-hydroxy-3-methylglutaryl-co-enzyme A (HMG-CoA) reductase thus promoting cholesterol biosynthesis (Palmer et al., 1997). Therefore, the ability of P. mildbraedii and M. flagellipes to decrease TC, TG, and LDL could be attributed to the inhibition of HMG-CoA reductase or activation of lipoprotein lipase thereby opposing the mechanism of action of P407 which was used to induce hyperlipidemia in the rats.

The study results imply that P. mildbraedii and M. flagellipes could be effective agents in the treatment of hyperlipidemia and risk factors associated with it such as coronary heart disease by decreasing lipid levels (Mozaffarian et al., 2015). Their ability to decrease LDL levels means they could serve as important agents in reducing one's risk of atherosclerosis since LDL level is its most important singular marker of atherosclerosis (Banyopadhyay et al., 2018). The n-hexane fractions of P. mildbraedii and M. flagellipes had no significant change on the HDL-C levels, but decreased the CHOL/HDL-C ratio of the rats. The CHOL/HDL-C ratio is important in diagnosis of heart diseases such as ischemic heart disease and acute myocardial infarction (Calling et al., 2019) and thus lowering its ratio could help in reducing one's risk of heart diseases.

5. Conclusion

In summary, this study has revealed that the n-hexane fraction of M. flagellipes and P. mildbraedii have inhibitory activity against pancreatic lipase and alpha-amylase in vitro, and thus have the potential to serve as anti-obesity agents via the mechanism of inhibition of digestive enzymes. Also, the in vivo studies revealed that P. mildbraedii and M. flagellipes has antihyperlipidemic properties.

Author contributions

Dr. Gabriel Anyanwu conceptualized and supervised this study, Dorathy Anzaku and Chinda Donwell performed the experiments in the laboratory, Dr. Gabriel Anyanwu, Dorathy Anzaku, Usunobun Usunomena, Muyiwa Adegbegi and Patricia Ofoha participated in the data analysis and drafting of the manuscript, while Dr. Khalid Rauf reviewed and edited it. All data were generated in-house, and no paper mill was used. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of Competing Interest

All authors have no interest to declare.

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