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ANTICHOLESTEROL ACTIVITY OF *AJU MBAISE*

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ABSTRACT

Obesity is a growing health concern worldwide and is referred to as the 'menace of obesity'. It is linked to various diseases and can lead to a decrease in quality of life. This study focuses on the use of the Aju Mbaise, a local herb combination, in aiding weight loss. Also, one of the objectives of this study is to determine the component or components responsible for the Aju Mbaise weight loss activity. Thirty-two rats of both sexes were used and divided into eight groups. The first group was fed with standard diet as the negative control. The last group was dosed with atorvastatin as the positive control and the rest groups of rats were dosed with Aju Mbaise and its component parts. Phytoconstituent extraction of the Aju Mbaise was carried out using methanol and dichloromethane as solvents. Phytochemical screening and GCMS analysis was undertaken to determine the classes of compounds contained in the Aju Mbaise. Serological testing to determine plasma levels of High Density Lipoprotein, Low Density Lipoprotein, total cholesterol and triglycerides was carried out. The weights of the animals were monitored for 21 days. Also, GC-MS Analysis to determine the bioactive compounds was carried out. The data was analyzed by one-way analysis of variance and Duncan's Post Hoc test. There was marked weight loss by *Ceiba pentandra*, the mixture of *Barteria fistulosa* and *Napoleona vogelli* as well as *Uvaria chamae at P< 0.05*. GC-MS analysis revealed 7-Nonenoic acid, methyl ester, 8-Nonynoic acid and 2-Piperidinone, N-[4-Bromo-n-butyl]- which have been reported for antibacterial activity. Additionally, 8-Nonynoic acid is known to inhibit the synthesis of fatty acids. Serological results showed optimum levels of Triglycerides and Very Low Density Lipoprotein, while plasma levels of High Density Lipoprotein, Low Density Lipoprotein and total cholesterol were moderate.

KEYWORDS: Aju Mbaise, Obesity, weight – loss.

1.0 INTRODUCTION

The problem of obesity has grown to epidemic proportions, with over 4 million humans losing their lives each year as a result of being overweight or obese in 2017 in line with the global burden of disease. Rates of overweight and obesity keep growing in adults and kids. From 1975 to 2016, the prevalence of overweight or obese kids and obesity are now dramatically on the upward thrust in low- and middle-earnings countries, specifically in city settings. The substantial majority of overweight or obese children live in developing countries, where the rate of growth has been greater than 30% higher than that of advanced countries, overweight and obesity are now dramatically on the upward thrust in low- and middle-earnings countries, specifically in city settings.[1] The substantial majority of overweight or obese children live in developing countries, where the rate of growth has been greater than 30% higher than that of advanced countries. There are many reasons why some people have difficulty losing weight. Usually, weight problems result from inherited, physiological and environmental factors, mixed with diet, physical activity and workout choices.[2]

According to study carried out in 2021, the prevalence of overweight and obesity in Nigeria is estimated to be 27.6% and 14.5%, respectively. Men had an overweight prevalence of 26.3% while women had 28.3%. The obesity prevalence among men was 10.9% and 23.0% among women respectively. Geographically, the highest prevalence of overweight was in the Southeast (29.3%), followed by Southwest (29.3%), South-south (27.9%), Northwest (27.2%), North-central (25.3%), and Northeast (20%). The highest prevalence of obesity was in the South-south (24.7%), followed by Southeast (15.7%), Southwest (13.9%), Northwest (10.4%), North-

central (10.2%) and Northeast (6.4%). It is evident that the southern geopolitical zones have a higher prevalence of overweight/obesity than other zones in Nigeria overall, making this a public health concern.[3]

According to yet another study carried out, it was found that roughly 25% of Nigerians are overweight and 14.3% are obese. Women had a higher prevalence of overweight and obesity than men at 25.5% and 19.8% respectively. The mean BMI and waist circumference were 25.6kg/m2 and 86.5cm respectively. According to our estimates, about 21 million people in Nigeria were overweight, with 12 million being obese in 2020 – approximately 20% and 11.6%, respectively, age-adjusted. Urban dwellers had higher rates of overweight and obesity than rural residents (27.2% and 14.4% compared to 16.4% and 12.1%). These figures indicate a high prevalence of overweight and obesity, especially in urban areas and among women, which could be due to the trend of sedentary lifestyles combined with the abundant availability of processed food across many African locations. Nutritional shifts triggered by demographic changes, rising income, urbanization, unhealthy habits, as well as consumption of heavily processed food are largely responsible for driving the obesity epidemic across Nigeria.[4]

Public health professionals warn that unless immediate steps are taken collectively and individually, obesity could create a health crisis. Doctors have noted that while the coronavirus can affect anyone, people who are obese are more at risk of developing serious symptoms. This is largely due to abdominal fat pushing the diaphragm up and reducing lung capacity, as well as being linked to comorbid conditions like diabetes and heart disease, which then leads to increased inflammation and weakened immunity. Sadly, this pandemic is still causing more individuals to become obese due to insufficient outdoor activities and lack of access to weight loss programs. To protect against weight gain, nutritionists suggest consuming plenty of fruit, vegetables, and grains plus exercising for at least an hour per day.[5]

The connection between humans and their use of medicinal plants dates back to ancient times, with evidence ranging from written documents to preserved monuments and original plant medicines. This knowledge has grown over time, allowing pharmacists and doctors to better understand and cope with modern illnesses. Initially, people discovered remedies through trial and error in the absence of any reliable information. Over time, they gained a better understanding of which plants to use for what ailments, transitioning from an empirical approach to one based on facts. Prior to the introduction of iatrochemistry in the 16th century, plants were the primary source for treatments and prevention. Nowadays, their effectiveness is re-emerging due to the limited efficacy and side effects of synthetic drugs.

The use of herbal plants is now widespread because of their availability, ease of use, ease of accessibility, and due to the fact that they are usually extremely affordable. Some plants that have been known to assist in weight loss includes Black pepper, Ginger, Cinnamon, Ginseng, Chilli pepper, Aju Mbaise and a host of others too. After careful examination, it has been seen that Aju Mbaise has been proven to be one of the most used and sort after remedies and this study will be centred on examining its weight loss properties. The Aju Mbaise herb got its name from it producers, a village called Mbaise, in Imo state, Nigeria. It is an Orthodox method of trimming down post pregnancy fat by the Mbaise women when cooked with uda, uziza and ehuru. Aju mbaise wasn't sold in Mbaise those days because almost all the women of child bearing age knew the bush to get it from.

It was Introduced to the local Igbo markets when they started inter marrying from other Igbo communities. So, Aju Mbaise started its journey of circulation in some Nigerian market as some indigenes even travel abroad with it when they saw the significant results. While the herbs work effectively, the fake ones also filtered into the markets.

Aju Mbaise has proven effective for overall weight loss and flattening of the stomach especially after childbirth most achievable within 3- 4 weeks of intensive use.

Aju mbaise is a fat burning traditional herb is a combination of 6 different leaves, roots and bark of a medicinal tree wrapped together: *Ceiba pentandra*, *Barteria fistulosa*, *Napoleona vogelli*, *Uvaria chamae*, E*uphorbia convolvuloids* and *Spondias mombin*.

Barteria fistulosa

The roots, bark, and leaves of this tree are used to aid in weight loss, treat fevers and pains; a decoction of the bark has many applications; the sap aids in wound healing; some portions of the tree are used to treat epilepsy and snake bites; and the young shoots are said to have aphrodisiac powers[6] (Umberto, 2016).

Euphorbia convolvuloids

In ethno pharmacology, *Euphorbia convolvuloids* are used to treat insect bites, diarrhea, dysentery, and respiratory tract infections. It is also an essential component of Aju Mbaise and is therefore believed to have anticholesterol activity.

Napoleonae vogelli

The most typical uses for *Napoleona vogelii* in herbal remedies are to treat burns, wounds, fever, pneumonia, syphilis, and staphylococcal infections[7] (Adegoke et al, 2001). A study from the Tropical Journal of Pharmaceutical Research also found that the leaves had hypolipidemic and hypoglycemic effect.

Spondias mombin

Spondias mombin is the scientific name for it. It is a plant with several traditional uses. The leaf is frequently used to start labour in conventional birthing procedures. On wounds and inflammatory locations, poultices made of the juice of the crushed leaves and the powder of the dried leaves are applied. In the traditional medicine of Africa and Latin America, extracts of *Spondias mombin* L. (Anacardiacea) are used to treat a wide range of inflammatory conditions. The leaf is frequently used to start labor in conventional birthing procedures. On wounds and inflammatory locations, poultices made of the juice of the crushed leaves and the powder of the dried leaves are applied. Its anticholesterol activity is still under investigation and it is therefore one of the aims of this study to find that out.[8]

Ceiba pentandra

Indigenous medicine *Ceiba pentandra* (Bombacaceae) is also referred to as Sweta Salmali in Ayurveda. The bark is useful in treating hepatopathy and vitiated conditions of the vata and kapha doshas. It is acrid, bitter, thermogenic, diuretic, emetic, purgative, and tonic. Decoction of the bark of *Ceiba pentandra* has also been used to treat type II diabetes, headaches, and as an aphrodisiac. It is a component of several Ayahuasca drinks that contain hallucinogenic ingredients.[9]

Uvaria chamae

Uvaria chamae, often known as finger root or bush banana, is a tropical West and Central African climbing big shrub or small tree that thrives in wet and dry woods and coastal scrublands. The fruit growing in its little bunches is referred to by its common name; the fruit is palatable and commonly consumed. *U. chamae* is a medicinal plant with antibiotic properties that is used to treat fevers throughout its range. A *Uvaria chamae* extract exhibited considerable antimalarial action against both early and established infections when given orally at 300–900 mg/kg/day.[10]

Aju Mbaise means “a wrap from Mbaise” and it is given to nursing mothers to help them regain nutrients lost during childbirth as well as assist in general weight loss and trimming of belly fat.

The aim of this study is to determine the anticholesterol activity of the various components of Aju Mbaise.

2.0 MATERIALS AND METHODS

2.1 COLLECTION AND IDENTIFICATION OF PLANT MATERIALS

Wraps of Aju Mbaise plant were purchased from the oil mill local market in Port Harcourt, Rivers State. The wraps were separated into its component parts and identified and authenticated by the Department of Plant Science and Biotechnology, University of Port Harcourt.

2.2 PHYTOCONSTITUENT EXTRACTION

Extracts were prepared both as a unit and for the individual plant components. To achieve this, the plant materials were air dried for one week and then ground to coarse powder using a petrol powered milling machine. Extraction was done using equal parts of methanol and dichloromethane as extraction solvents. Using a ratio of 3 to 1 for solvent to amount of powdered plant materials, equal parts of both methanol and Dichloromethane were added to the pulverized plant materials and then macerated for 72hours. This was then filtered using a filter and further finely filtered using a filter paper. The different extracts were then concentrated using a rotary evaporator and then further subjected to evaporation using a water bath.

2.3 EQUIPMENTS/INSTRUMENTS

- Beaker
- Filter paper
- Measuring cylinder
- Thermostat water bath
- Electronic weighing balance
- Evaporating dish
- Sample bottles
- Funnel
- Cotton wool
- Mechanical grinder
- Refrigerator
- Spatula
- Sieve.

2.4 CHEMICALS/REAGENTS

- Methanol (analytical grade)
- Dichloromethane (analytical grade)
- Dragendorff’s reagent
- Mayer’s reagent
- Ferric chloride solution
- Hydrochloric acid
- Wagner’s reagent
- Olive oil
- Molish reagent
- Picric acid
- Million’s reagent
- Fehling’s solution
- Aqueous Sodium hydroxide
- Benzene
- Acetic anhydride
- Kedde’s reagent
- Dilute Sulfuric acid
- Distilled water.

2.5 EXPERIMENTAL ANIMALS

The experiment was done using 32 Wistar rats weighing 100-175 g of both sexes. They were purchased from the Department of Pharmacology and Toxicology, Animal House, Basic Medical Sciences, University of Port Harcourt and kept in an environmental friendly conditioned polypropylene cages with unrestricted access to food (regular pellets) *ad libitum* and clean drinking water. Temperatures and humidity were maintained at 24

± 1°C and (50 ± 5 %) on a 12 hrs light-dark cycle. The animals were kept together for 7 days before the experiment was conducted to allow for acclimatization. Experimental protocol of the international accepted principle on laboratory animal use, care and handling was adhered to completely.

The rats were divided into eight groups for different treatments. The First group was treated with only standard diet (negative control). The Second group was treated with the extract labelled as "Others", the Third group was treated with the stock labelled Aju Mbaise. The Fourth group was treated with the stock labelled *Euphorbia convolvuloids* and the Fifth group was treated with the stock labelled *Ceiba pentandra*. The Sixth group was treated with the stock labelled Mixture (which is a mixture of *Barteria fistulosa* and *Napoleona vogelli* leaves).

The Seventh group was treated with extract labelled *Uvaria chamae* while the Eighth group was treated with Atorvastatin. The strength of the stock solution of the extracts were 100mg/ml and the dose administered was according to 200mg/kg of body weight. The dosing was carried out for a period of 21 days and results were recorded accordingly.

2.6 PHYTOCHEMICAL SCREENING

Preliminary phytochemical screening was carried out on all the crude leaf extracts (methanol/dichloromethane) of Aju Mbaise using standard procedures as described by Trease and Evans[11], Sofowora et al.,[12], Ushie et al.,[13] and Santhi et al.[14]

2.7 STATISTICAL ANALYSIS

The tests were performed in quadruplicates. Mean and Standard deviation (M±SD) were calculated and 95% confidence interval (CI) of means was used. To compare between groups, analysis of variance (ANOVA) were calculated. *P*-values of less than 0.05 were considered as statistically significant. All statistical analysis was performed using Graph pad prism version 8.0 software.

2.8 SAMPLE PREPARATION FOR LIPID PROFILE TEST

After the treatment, 1.5ml of blood from the orbital vein were collected in tubes containing EDTA (heparinized tubes). The samples were centrifuged at 3000 rpm for 10mims and the plasma were used for measuring the total cholesterol, LDL- cholesterol, HDL-cholesterol, VLDL-cholesterol and triglycerides level. The total plasma cholesterol and triglycerides were measured according to the instruction manual accompanying the diagnostic kits from the Lively Stones Medical Diagnostic Laboratory. High density lipoprotein (HDL) cholesterol, Low density lipoprotein (LDL) cholesterol were measured according to the aforementioned instruction manual too.

2.9 GC-MS ANALYSIS AND CONDITIONS

The gas chromatography mass spectrometry (GC-MS) analysis of the crude methanol/dichloromethane extract of Aju Mbaise was quantitatively determined using an Agilent 7890B GC system coupled with an Agilent 5977A MSD with a Zebron-5MS column (ZB-5MS 30 m × 0.25 mm × 0.025 µm) (5%-phenylmethylpolysiloxane). The GC-grade helium served as the carrier gas at a constant flow rate of 2 mL/min. The crude extract was dissolved with ethanol and filtered before use. The column temperature was maintained at 60°C and gradually increased at 10°C per minute until a final temperature of 300°C was reached. The time taken for the GC-MS analysis was 30 min. The compounds were identified based on computer matching of the mass spectra with the NIST 11 MS library (National Institute of Standards and Technology library).

3.0 RESULTS

Table 1: Phytochemical screening results of aju mbaise extract.

S/N	Phytochemical constituent	Results
1	Alkaloids	Positive
2	Tannins	Positive
3	Saponnins	Negative
4	Flavonoids	Negative
5	Anthraquinone	Negative
6	Phlobatannins	Positive
7	Steroids	Negative
8	Tritepenoids	Positive
9	Carbohydrates	Positive
10	Protein	Positive

Table 2: Results of serological testing.

GROUPS	TC	TG	HDL	LDL	VLDL
CONTROL	2.6±0.2	1.3±0.1	1.4±0.2	1.8±0.1	0.6±0.0
OTHERS	2.6±0.1	1.0±0.1	1.3±0.1	1.8±0.2	0.5±0.0
AJU MBAISE	2.6±0.2	1.1±0.1	1.1±0.1	2±0.2	0.5±0.0

EUPHORBIA CONVOLVULOIDS	2.7±0.2	1.1±0.1	1.2±0.0	2±0.2	0.5±0.1
CEIBA PENTANDRA	3.2±0.2	1.4±0.1	1.5±0.2	2.3±0.1	0.6±0.0
MIXTURE (A combination of *Barteria fistulosa* and *Napoleona vogelli*)	2.6±0.2	1.1±0	0.9±0.0	2.1±0.2	0.5±0.0
UVARIA CHAMAE	2.8±0.2	1.2±0.1	1.3±0.1	2.0±0.3	0.5±0.0
ATORVASTATIN	2.5±0.2	1.6±0.7	1.3±0.2	1.6±0.1	0.9±0.5

The results of the weight monitoring of the rats are presented in figure 9.

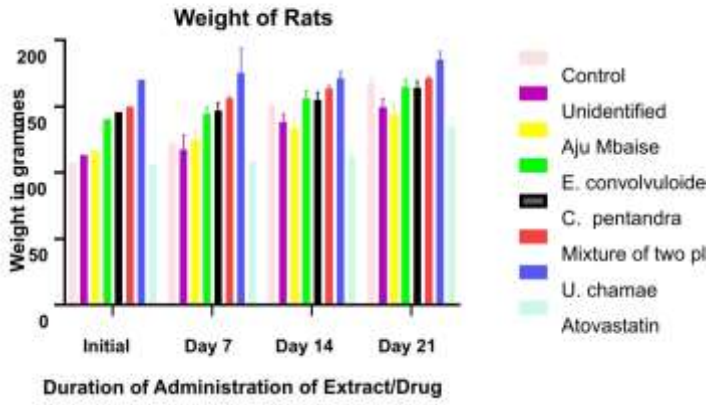


Figure 1: A Bar Chart Showing the progressive weight gain of Wistar rats.

The bar chart displayed above shows the progressive weight gain of the Wistar rats over the course of the experiment. It can be clearly deduced there was a progressive increase in weight as the weeks progressed. This was due to the fact that no form of fasting was implemented as the rats were fed every day for the period of the experiment. Weight gain however across different groups also differed.

An analysis of the Percentage Weight gain of the Wistar rats are shown in figure 2.

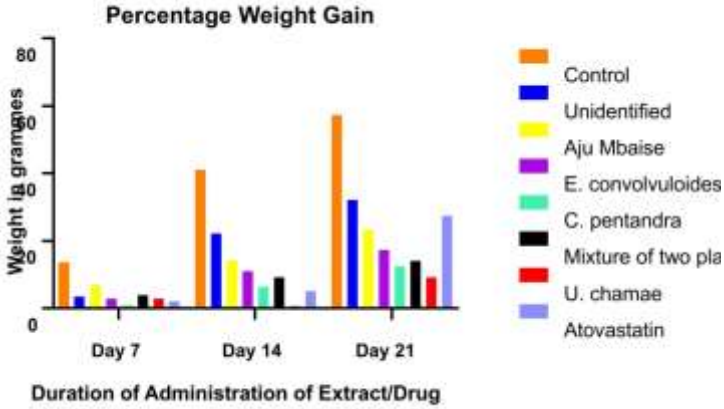


Figure 2: A Bar chart Showing the percentage weight gain of Wistar rats.

Figure 2 shows the percentage weight gain observed as the experiment progressed through the weeks. It gives a clearer picture as to what happened as the weeks went by during the experimental procedure. It was observed that by the second and third week, weight gain in the groups treated with *Ceiba pentandra,* mixture of *Barteria fistulosa* and *Napoleona vogelli* as well as *Uvaria chamae* were seen to have remarkable controlled weight gain when compared to both control groups. This supports the acclaimed weight-loss activity of Aju Mbaise.

There was significant difference in the weight loss of *Uvaria chamae* at day 14 and 21, at $p<0.001$ when compared to control, then $p<0.05$ when compared to Aju Mbaise and Atorvastatin. This statistical analysis result goes further to support the results of the serological testing were *Uvaria chamae* was seen to control the tested parameters just as good as Atorvastatin. Therefore, it shows that Aju Mbaise is effective in controlling not only weight, but also the aforementioned tested parameters.

GC-MS analysis was carried out to identify the likely non-polar compounds in the Aju Mbaise and the chromatogram is presented in Figure 3.

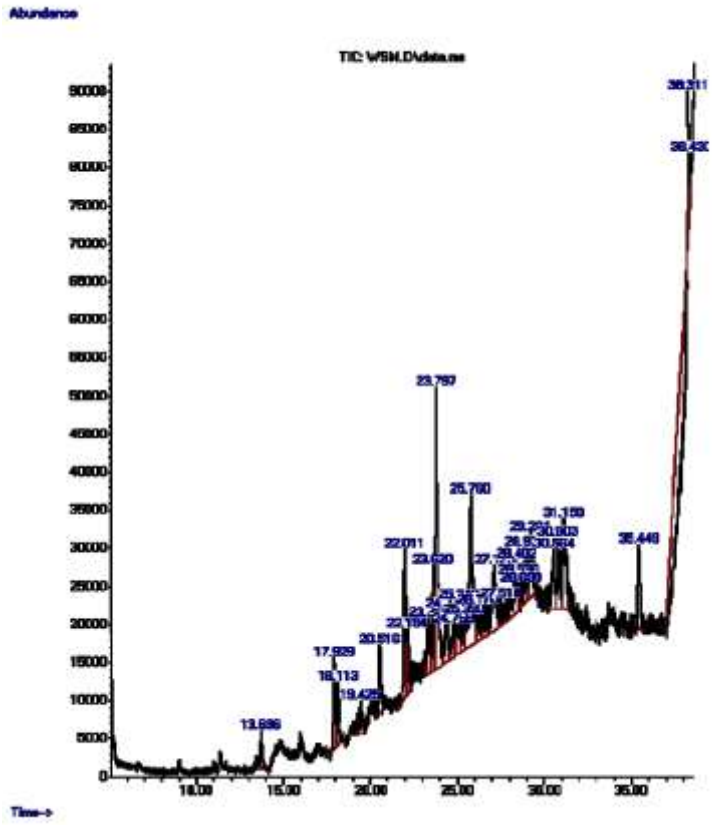


Figure 3. A chromatogram Showing result obtained from GC-MS analysis.

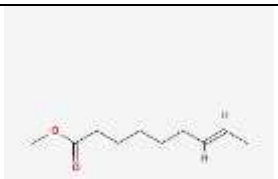
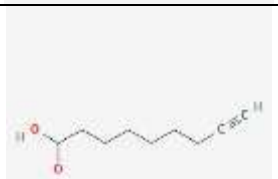
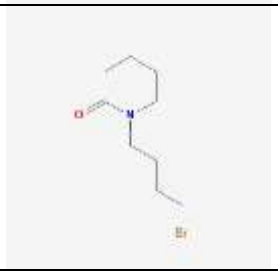
Table 3: Showing compounds identified from gc-ms analysis.

Peak	Retention time	Area percentage	Library/ID Reference
2	17.928	3.960	Oxalic acid, cyclobutyl tridecyl ester
5	20.516	3.966	**7-Nonenoic acid, methyl ester**
10	23.797	19.984	9,12-Octadecadienal
15	25.759	12.915	Oxalic acid, allyl hexadecyl ester
16	26.177	2.240	**8-Nonynoic acid**
19	28.402	1.135	Hexadecanal
20	28.529	7.298	Hexadecanal
23	29.204	4.652	**2-Piperidinone, N-[4-**

			bromo-n-butyl]-
27	35.448	5.359	Succinic acid, di(3-methylbut-3-enyl) ester

The GC-MS analysis results revealed the presence of certain compounds which were of significance. The compounds were 7-Nonenoic acid, methyl ester, 8-Nonynoic acid and 2-Piperidinone, N-[4-bromo-n-butyl]- which possess antibacterial activities.[15,16,17] 8-Nonynoicacid from studies, inhibits the growth of gram-negative bacteria through the inhibition of fatty acids synthesis. This action is akin to the role of Omega-3 fatty acids known to be good regulators of weight-gain and other metabolic activities. Also, piperine; a structural analogue of 2-Piperidinone, N-[4-bromo-n-butyl]- has also been observed in a study to control dyslipidemia in obesity-induced rats.[18]

Table 4: Showing compounds,their structure and analogues.

Compounds	Structure	Analogue compounds
7-Nonenoic acid, methyl ester		7-Tetradecenoic acid, (7Z)-
8-Nonynoic acid		11,14-Octadecadienoic acid, (11Z,14Z)-
2-Piperidinone, N-[4-bromo-n-butyl]-		5,8-Tetradecadienoic acid, (5Z,8Z)-

DISCUSSION

Phytochemical screening was carried out on the Aju Mbaise extract as a whole to determine the various phytoconstituents therein. Results obtained from the screening showed the confirmed presence of alkaloids, Tannins, Saponnins, Phlobatannins, Tritepenoids, Carbohydrates and Protein while Flavonoids and Steroids were absent.

The weight-loss experimental part of the research was carried out using 32 Wistar rats of both sexes. It was done to determine the component or components responsible for the weight-loss activity of Aju Mbaise. At the end of the experimental procedure, it was seen that the weights of the rats increased as the weeks went by, this was due to the fact that no form of fasting was carried out and the rats were adequately fed. However, on taking a closer look at the percentage weight gain bar chart, it was seen that the groups treated with extracts of *Ceiba pentandra*, mixture of *Barteria fistulosa and Napoleona vogelli* as well as *Uvaria chamae* showed remarkably less weight gain compared to the other groups. Out of the three aforementioned groups however, *Uvaria chamae* showed the most remarkable activity yet.

Obese individuals have been seen to have increased levels of Total cholesterol, Triglycerides, High density lipoprotein, Low density lipoprotein and Very low density lipoprotein when compared to healthy individuals. The serological testing results revealed optimum levels for Triglycerides and Very low density lipoprotein, while the rest parameters which were tested showed moderate levels. This also demonstrates the ability to control these parameters in obese individuals thereby improving state of health.

The GC-MS analysis carried out revealed quite a number of compounds of interest which were outlined in the

Table 4 above. These compounds have been seen to have bearing with omega-3 fatty acids. Omega-3 fatty acids are a type of polyunsaturated fatty acid with a double bond in the third carbon position from the methyl end. The most common omega-3 fatty acids are alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). These fatty acids have been associated with various health benefits, such as reducing inflammation and lowering the risk of heart disease.[17] Some of the compounds detected were reported to have antibacterial activities and even inhibit the synthesis of fatty acids.[15,16,17] This also goes further to scientifically support the already made claim that Aju Mbaise can be used for its weight-loss activity. From the study carried out, the results showed that Aju Mbaise possesses weight-loss activity and can therefore could be used in the management of weight loss.

CONCLUSION

In conclusion, the components *Ceiba pentandra*, mixture of *Barteria fistulosa* and *Napoleona vogelli* as well as *Uvaria chamae* were seen to possess weight-loss activity. Also, when compared to Atorvastatin, the extracts were seen to possess significant anticholesterol activities. The GC-MS analysis also revealed various Omega-3 analogues that may contribute to the weight-loss activity of Aju Mbaise.

Further study on the components of Aju Mbaise mentioned above will give a more detailed insight on their individual weight-loss activity as well as the specific compounds responsible for such activity.

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