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Plants Composition and Identification of Phytocomponents in a Polyherbal Formulation used in Southeast Nigeria

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ABSTRACT

A polyherbal formulation (Ajumbaise) used in southeast Nigeria was screened for its plants composition and presence of phytochemical substances by Gas Chromatography-Mass Spectrometry (GC-MS). Results obtained showed that the formulation is made up of six different plants in varying percentage compositions including *Barteria fistulosa* (34.97%), *Napoleona vogelli* (23.72%), *Euphorbia convolvuloids* (3.72), *Spondias mombine* (11.45%), *Uvaria chamae* (10.09%) and *Ceiba petandra* (16.60%). Extract yield for the polyherbal as a unit was 17.38% while for individual plants compositions the yields were *Barteri fistulosa* (32.40%), *Napoleona vogelli* (31.60%), *Euphorbia convolvuloids* (15.00%), *Spondias mombine* (29.00%), *Uvaria chamae* (10.20%) and *Ceiba petandra* (7.72%).Preliminary phytochemical analysis of the polyherbal extract revealed the presence of flavonoids, steroids, terpenes, phenolic compounds and alkaloids in high quantities. Saponins and tannins were present in moderate quantities while glycoside was found in small quantity. GC-MS chromatogram of the polyherbal extract showed sixteen peaks indicating presence of sixteen phytochemical constituents including Methyl (2)-3-cyanoprop-2-enoate (1.22%), 2-Ethyl-2-hexen-1-al (0.80%), 1,3-oxazolidine-2-thione (2.54%), Benzyl benzoate (2.71%), Methyl 2-(4-chlorophenoxy)-2 methyl propanoate (5.00%), Hexa decanoic acid also known as Palmitic acid (32.65%), Ethyl palmitate (6.74%), S-methyl-L-cysteine (3.49%), Niacin or nicotinic acid (6.96%), *N*-(furan-3-yl) acetamide (10.60%), Stearic acid or n-Octadecanoic acid (12.34%), and Pyridine-4-carboxylic acid (1.94%). Others are Pyroglutamic acid (0.91%), Pyroglutamic acid (0.91%), ϱ-nitrocinnamic acid, methyl ester (1.42%), 17-carboxyheptadec-9-en-1-ylium (7.10%) and 20-carboxydodec-8-en-1-ylium (3.58%). We therefore conclude that Ajumbaise polyherbal formulation is made up of 6 different medicinal plants and is heavily enriched with phytocomponents which may be of value as antioxidants, hypolipidaemic agents and in the prevention and management of cardiovascular diseases.

Keywords: GC-MS, Phytochemical, Polyherbal, Antioxidants

INTRODUCTION

Africa is indeed heavily endowed with abundant natural resources and is home to millions of medicinal plants. This, in addition to cost and accessibility may be the reason why majority of its dwellers rely on medicinal plants for the management of diseases (Sofowora, 1993). Today, the use of medicinal plants has assumed a global perspective. It is reported that about 88% of the world's population is relying on herbal medicine (Marshin *et a*l., 2013; Perera *et al*., 1985). This keen interest in herbal medicine has of late stirred up numerous research interests in the world of science. These researches in most cases are aimed at evaluating the pharmacological activities and identification of bioactive substances responsible for such activities. These researches have also become a major link between herbal and orthodox medicines and have over the years mediated between the two practices. Recent reports have shown that orthodox medicine is strongly anchored on traditional medicine and has always provided information on the needed research area for new drug discovery (Ojieh *et al*., 2013).

In southeast, Nigeria, herbal medications are prepared as infusions, concoctions or decoctions of a part or parts of single or more plants. The polyherbal formulation (Ajumbise) is a combination of the leaves, stems, bark and roots of different species of plants put together in various proportions. The formulation is native to the Mbaise Community in Mbaise Local Government area of Imo State, Nigeria. The virtual non existence of written information on this herbal preparation limited the sources for this section of our work to interviews and personal observations. According to these natives, the formulation which usually is taken as an infusion is used for various purposes including, enhancing labour, facilitating the removal of placenta after delivery, relieving menstrual and post-delivery pain and promoting involution of the uterus after delivery. These all require scientific validations which we did, but in this current publication, we present in compressive terms the plants composition, preliminary phytochemistry and identified GC-MS phytocomponents of the herbal as our other findings follow in our other publications.

Plate 1.1: Heads and a single head of fresh Ajumbise polyherbal formulation purchased from Onu-imo herbal market, Obowo Local Government Area, Imo state, Nigeria

MATERIALS AND METHOD

Collection and Identification of Plant Material

Heads of *Ajumbise* polyherbal were purchased from Onu-imo herbal market in Obowo Local Government Area of Imo State, Nigeria. Some were separated into its component plants and were identified at the Department of Forestry, College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike. The identified plants were assigned voucher numbers and specimens were preserved in the herbarium of the Department of Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike.

Preparation of Plant Extract

Extracts were prepared for the Polyherbal as a unit and also for the individual plant components. To achieve this, the plant materials were air dried at room temperature for 21 days and then ground to coarse powder using a locally fabricated milling machine powered by a petrol motor (Honda Company, Japan). For each round of extraction, Fifty (50) grams of the powdered material was introduced into the extraction chamber of the soxhlet extractor and extraction was done using ethanol as solvent. Extraction temperature was maintained at 60^0C for 48 hours. At the end of the period, the ethanol was evaporated at low temperature in a hot air oven to obtain crude extract which weighed 8.69g and represented a percentage yield of

17.38% for the Polyherbal and varying extract yields for the separate components (Table 1). The extracts were preserved in the refrigerator until needed. The extract of the polyherbal formulation is hereafter referred to as Ajumbise Polyherbal Extract (APE).

Preliminary Phytochemical Investigation

Preliminary Phytochemical studies was carried out according to the methods described by Trease and Evans, (1989) and Harborne, (1973) and used by Deka and Kalita, (2012).

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of APE

The characterization of the Phytochemicals in APE was done using GC-MS QP2010 Plus (Shimadzu, Japan) while the identification of the phytochemicals in the sample was carried out using a QP2010 gas chromatography with Thermal Desorption System, TD 20 coupled with Mass Spectroscopy (Shimadzu). The ionization voltage was set at 70eV. Gas Chromatography was conducted in the temperature programming mode with a Restek column (0.25 mm, 60 m, XTI 5).The initial column temperature was 80^{o}C for 1min, and was later increased linearly at 70^{o}C min-1 to 220^{o}C, held for 3 min followed by linear increased temperature 10oC min-1 to 290^{o}C for 10 minutes. The temperature of the injection port was 290^{o}C and the GC-MS interface was maintained at 290^{o}C .The sample was introduced via an all-glass injector working in the split mode, with helium carrier gas low rate of 1.2 ml min-1. Identification of compounds was accomplished by comparison of retention time and fragmentation pattern, as well as with mass spectra of the GC-MS. Identity of the active components in the extract was by comparison of their retention indices, peak area percentage and mass spectra fragmentation pattern with those stored on the National Institute of Standards and Technology (NIST) digital library data and also with published Literature. NIST08.LIB, (Stein, 1990), WILEY 8 LIB, (Lafferty, 1986), library sources was used for matching the identified components from the plant material. With that the name, molecular weight, formula, structure and bioactivities of the compounds were then ascertained.

RESULTS

Plants Composition and Extract Yields

Separation and individual identification of the plant components present in polyherbal formulation revealed the presence of six different plants in different compositions. The plants include *Barteria fistulosa* (34.97%), *Napoleona vogelli* (23.72%), *Euphorbia convolvuloids* (3.72), *Spondias mombine* (11.45%), *Uvaria chamae* (10.09%) and 16.60% of *Ceiba petandra* (Table 1). Extract yield for the polyherbal as a unit was 17.38% while for the plants compositions they yields were *Barteria fistulosa*(32.40%), *Napoleona vogelli* (31.60%), *Euphorbia convolvuloids* (15.00%), *Spondias mombine* (29.00%), *Uvaria chamae* (10.20%) and 7.72% for *Ceiba petandra* (Table 1)

Table 1: Plants compositions of Ajumbise Polyherbal and Extract yields

S/N	BOTANICAL NAME AND FAMILY	COMMON NAMES	PLANT PART USED	PERCENTAGE COMPOSITION	PERCENTAGE EXTRACT YIELD
1	*Barteri fistulosa (Passifloraceae)*	Igbo (Oje), Edo (Ogeimi), Yoruba (Oko)	Leaves	34.97	32.40
2	*Napoleona vogelli (Lecithidaceae)*	English (African nut tree), Igbo (Nkpodanwaoba),	Leaves	23.72	31.60
3	*Euphorbia convolvuloids (Euphorbiaceae)*	Igbo (Egele),	Whole plants	3.72	15.00
4	*Spondias mombine (Anacardiaceae)*	English (Hog plum), Igbo (Ichikara), Hausa (Isada), Yoruba (Iyeye)	Leaves	11.45	29.00
5	*Uvaria chamae (Annonaceae)*	Igbo (Mmimiohia), Hausa (Atore), Yoruba (Eruju)	Stem	10.09	10.20
6	*Ceiba petandra (Malvaceae)*	English (Cotton tree), Igbo (Akpuogwu), Hausa (Rimi), Yoruba (Araba)	Bark	16.60	7.72

Plate 1: ***Barteria fistulosa*** **leaves**

Plate 2: ***Napoleona vogelli*** **leaves**

Plate 3: ***Euphorbia convolvuloids*** **whole plant**

Plate 4: ***Spondias mombine*** **leaves**

Plate 5: ***Ceiba pentandra*** **bark**

Plate 6: ***Uvaria chamae*** **stems**

Preliminary Phytochemical Composition of APE

Results of preliminary phytochemical analysis of APE revealed the presence of flavonoids, steroids, terpenes, phenolic compounds and alkaloids in high amounts. Saponins and tannins were present in moderate amounts while glycoside was found in small quantity (Table 2)

Table 2: Results of Preliminary Phytochemical Components in APE

Phytochemical agent	Inference	Amount present
Saponins	++	Moderate
Flavonoids	+++	High
Tannins	++	Moderate
Steroids	+++	High
Terpenes	+++	High
Alkaloids	+++	High
Glycoside	+	Low
Phenolic compounds	+++	High

Results of preliminary gas chromatography-mass spectrometry (GC-MS) of APE

GCMS chromatogram of the ethanolic extract of APE (Figure 1) showed sixteen peaks which indicated the presence of sixteen phytochemical constituents. Figure 4.2 shows the mass spectra of the phytocomponents in the extract as were compared with that in the NIST Library database, leading to the identification and characterization of sixteen phytocomponents. The identified components and quantities found include Methyl (2)-3-cyanoprop-2-enoate (1.22%), 2-Ethyl-2-hexen-1-al (0.80%), 1,3-oxazolidine-2-thione (2.54%), Benzyl benzoate (2.71%), Methyl 2-(4-chlorophenoxy)-2 methyl propanoate (5.00%), Hexadecanoic acid also known as Palmitic acid (32.65%), Ethyl palmitate (6.74%), S-methyl-L-cysteine (3.49%), Niacin or nicotinic acid (6.96%), *N*-(furan-3-yl) acetamide (10.60%), Stearic acid or n-Octadecanoic acid (12.34%), and Pyridine-4-carboxylic acid (1.94%). Others are Pyroglutamic acid (0.91%), Pyroglutamic acid (0.91%), ǫ-nitrocinnamic acid, methyl ester (1.42%), 17-carboxyheptadec-9-en-1-ylium (7.10%) and 20-carboxydodec-8-en-1-ylium (3.58%) (Table 3).

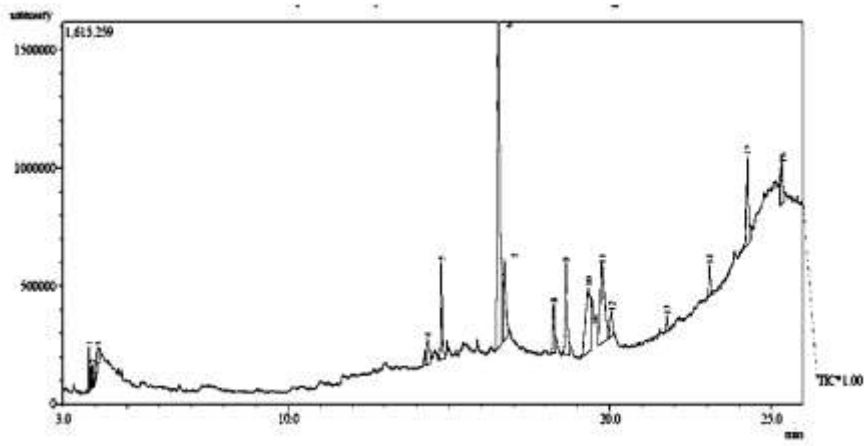
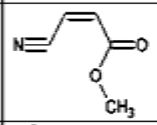
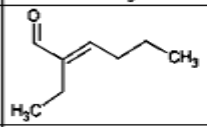
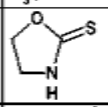
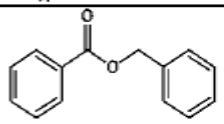
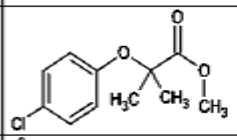
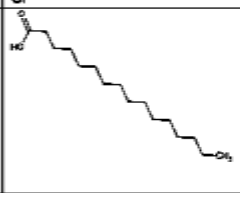
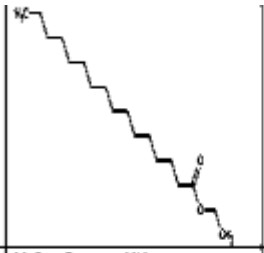
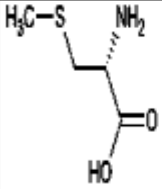
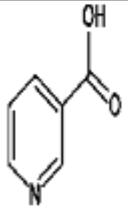
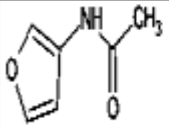
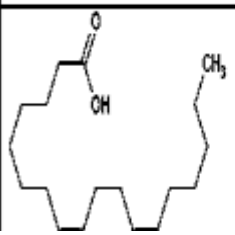
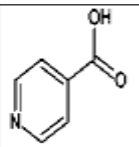
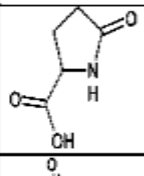
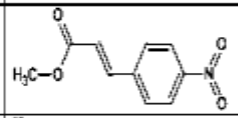
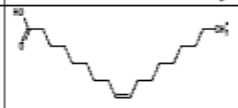
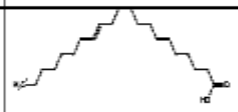


Figure 1: Showing 16 peaks obtained following GC-MS analysis of APE

Table 3: Phycomponents in APE

S/No	Name of Compound	Retention time	Peak area %	Molecular weight	Molecular formular	Molecular structure	Bioactivity
1	Methyl (2Z)-3-cyanoprop-2-enoate	3.823	1.22	111.09	$C_5H_5NO_2$		Increase Zinc Bioavailibility, Provide Zinc, Catechol-O-Methyl-Transferase-Inhibitor, Methyl-Guanidine-Inhibitor
2	2-Ethyl-2-hexen-1-al	3.930	0.80	126.19	$C_8H_{14}O$		5-Alpha-Reductase-Inhibitor, Allergenic, Albuminurigenic, Alcohol-Dehydrogenase-Inhibitor, Aldehyde-Oxidase-Inhibitor, Aldose-Reductase-Inhibitor, Alexiteric, Allelopathic, Algogenic (pain-causing)
3	1,3-oxazolidine-2-thione	4.115	2.54	103.14	C_3H_5NOS		Not found
4	Benzyl benzoate	14.333	2.71	212.24	$C_{14}H_{12}O_2$		Not found
5	Methyl 2-(4-chlorophenoxy)-2-methylpropanoate	14.760	5.00	228.67	$C_{11}H_{13}ClO_3$		Catechol-O-Methyl-Transferase-Inhibitor, Methyl-Donor, Methyl-Guanidine-Inhibitor
6	Hexadecanoic acid also known as Palmitic acid	16.531	32.65	256.42	$C_{16}H_{32}O_2$		Acidifier, Acidulant, Arachidonic acid, Arachidonic-Acid-Inhibitor, increase Aromatic Amino Acid Decarboxylase Activity, Inhibit Production of Uric Acid

7	Ethyl palmitate	16.738	6.74	284.47	$C_{18}H_{36}O_2$		Urine-Acidifier, Urinary-Acidulant, Inhibit Production of Uric Acid, Increase Aromatic Amino Acid Decarboxylase Activity, Arachidonic-Acid-Inhibitor,
8	S-methyl-L-cysteine	18.248	3.49	135.18	$C_4H_9NO_2S$		Low Sodium, 12-Lipoxygenase-Inhibitor, 5-Lipoxygenase-Inhibitor, Anti-LDL, Anticancer (Liver), Anticancer (Lung), Anticarcinogenic (Lung), Anti Corpus-Luteum, Antidote (Lead), Antidote (Lobelia), Antioxidant (LDL), Antitumor (Breast) (Lung) (Prostate), Antitumor (Liver), Antitumor (Lung), Benzodiazepine-Receptor Ligand
9	Niacin or nicotinic acid	18.639	6.96	123.10	$C_6H_5NO_2$		Anticancer (Oral), Antidote (organo-P), Antidote (Organophosphorus), Orexigen
10	N-(furan-3-yl)acetamide	19.332	10.60	125.12	$C_6H_7NO_2$		Anaphylactic (antidote-Neostigmine), Antitumor (Nasopharynx), Arylamine-N-Acetyltransferase-Inhibitor, Decrease Norepinephrine Production, GABA-nergic, Increase Natural Killer (NK) Cell Activity, Inhibit Production of Tumor Necrosis Factor, Myo-neuro-stimulant, N-Cholinolytic, Necrotizing, NCS-Depressant, Nauseant, Natriuretic, Narcotic, NADH-Ubiquinone-Oxidoreductase-Inhibitor, NADH-Oxidase-Inhibitor
11	Stearic acid or n-Octadecanoic acid	19.746	12.34	284.47	$C_{18}H_{36}O_2$		Urine-Acidifier, Urinary-Acidulant, Inhibit Production of Uric Acid, Increase Aromatic Amino Acid Decarboxylase Activity, Arachidonic acid-Inhibitor, Methyl-Guanidine-Inhibitor, Catechol-O-Methyltransferase-Inhibitor

12	Pyridine-4-carboxylic acid	20.043	1.94	123.10	$C_6H_5NO_2$		Urine-Acidifier, Urinary-Acidulant, Inhibit Production of Uric Acid, Increase Aromatic Amino Acid Decarboxylase Activity, Arachidonic acid-Inhibitor
13	Pyroglutamic acid	21.774	0.91	129.11	$C_5H_7NO_3$		Arachidonic acid-Inhibitor, Urine-Acidifier, Urinary-Acidulant, Inhibit Production of Uric Acid, Increase Aromatic Amino Acid Decarboxylase Activity,
14	p-nitrocinnamic acid, methyl ester	23.093	1.42	207.18	$C_{10}H_9NO_4$		Catechol-O-Methyl-Transferase-Inhibitor, Methyl-Guanidine-Inhibitor, Arachidonic acid-Inhibitor
15	17-carboxyheptadec-9-en-1-ylium	24.275	7.10	281.45	$C_{18}H_{33}O_2$		Decrease Endothilial Leukocyte Adhesion, Decrease Endothilial Platelet Adhesion, Encephalopathic, Endoanesthetic, Endocrinactive, Ergotamine-Enhancer, Enterotoxic, Enuretotic, Enterometility-Enhancer, Entendepressant, Entephalinogenic, Energizer
16	20-carboxydodec-8-en-1-ylium	25.327	3.58	323.53	$C_{21}H_{39}O_2$		Endothelium-Derived Relaxing Factor Promoter, Endothelium-Dependent, Enderphinogenic, Endocrinprotective, Endocrinactive, Endoanesthetic, Encephalopathic, Decrease Endothilial Platelet Adhesion, Decrease Endothilial Leukocyte Adhesion

DISCUSSION

Preliminary phytochemical studies of APE showed the presence of flavonoids, steroids, terpenes, phenolic compounds and alkaloids in high amounts, saponins and tannins in moderate amounts and glycoside in small quantity. These suggest that the herbal preparation is heavily enriched with bioactive healing substances. A higher number of plants components in Ajumbise polyherbal are green leafy plants and a number of scientific research have shown that most green leafy vegetables are rich in bioactive substances (Deka and Kalita, 2012; Saliu *et al*., 2012; Orhue *et al*., 2008;. Akindele and Busaya, 2011). The identified phytocomponents in APE indeed have been reported to be of medicinal values.

Flavonoids and other phenolic compounds are common dietary components present in many beverages and foods and have been reported to have antioxidant activity due to their free radical scavenging capacity. The consumption of flavonoids in food is also reported to be a means of preventing coronary heart diseases and management of cancer (Yao *et al*., 2004). Flavonoids also have been implicated in wound healing, cellular regeneration and

cytoprotection (Lewis *et al*., 1999; Kumar *et al*., 2013) and as such may be of benefit in ulcer management. Ntie-Kang *et al*., (2014) in their study on the anti-malarial effect of flavonoids suggested that the observed anti-malarial effect may have occurred due to ability of flavonoids to inhibit fatty acid biosynthesis of the parasite and the influx of L- glutamine and myoinositol into infected erythrocytes. Alkaloids are reported to contain large group of nitrogenous compounds which are widely used as cancer chemotherapeutic agents (Jioa *et al*., 2007; Jin-Jan *et al*., 2012). Many tannin components have also been reported to be anticarcinogenic and reduce mutagenic activity of a number of mutagens possibly due to their antioxidant property which prevents cellular oxidative damage including lipid peroxidation. Tannin also inhibits the generation of superoxide radicals and was found to possess strong antimicrobial activity (Chung *et al*., 1998; Huang *et al*., 2007). The ability of tannin to react with protein to provide a typical tannin effect which is important for the treatment of inflammatory or ulcerated tissues has also been reported (Parekh *et al*., 2005). Most plants that contain tannin as their main component have been used for treating intestinal disorders such as diarrhea and dysentery (Zhang, 2006). Steroids and terpenes increase protein synthesis, promote growth of muscles and bones and shows some level of antiviral activities (Huang*et al*., 2007). Saponins and glycosides are reportedly been used to alleviate cardiac problems associated with hypertension (Trease and Evans, 1985). Saponins in particular have been used to treat hyper cholesterolaemia in humans. This is because it is because it is believed to bind tocholesterol in the body to inhibit the reabsorption of the later thereby facilitating its excretion from the body.

GCMS analysis of APE showed the presence of Methyl (2)-3-cyanoprop-2-enoate, 2-Ethyl-2-hexen-1-al, 1,3-oxazolidine-2-thione, Benzyl benzoate, Methyl 2-(4-chlorophenoxy)-2 methyl propanoate, Hexadecanoic acid also known as Palmitic acid, Ethyl palmitate, S-methyl-L-cysteine, Niacin or nicotinic acid, *N*-(furan-3-yl) acetamide, Stearic acid or n-Octadecanoic acid, and Pyridine-4-carboxylic acid, Pyroglutamic acid, Pyroglutamic acid, ǫ-nitrocinnamic acid, methyl ester, 17-

carboxyheptadec-9-en-1-ylium and 20-carboxydodec-8-en-1-ylium.

Various reports on the medicinal values of these phyto substances exist. Furan based compounds are extensively reported to possess anti-cancer effects (Jane *et al* 1997**;** Byung *et al*; 2009; Erika, 2015). Niacin (Vit. B_3) is reported to have anticancer effect and lowers cardiovascular risks by boosting the levels of high density lipoprotein (HDL). There is evidence that niacin helps to lower atherosclerosis and the risk of Alzheimers disease, cataract, and osteoarthritis and type-1 diabetes. Other reported medicinal values of niacin are improving liver and digestive functions including carbohydrate and protein breakdown (Bradford, 2015; http/livesciences.com, 2015). Stearic acid is reported to possess antioxidant and anti-aging properties while results of clinical studies indicated that niacin was found to be associated with lowered low density lipoprotein (Hunter *et al*., 2009). A known toxic substance (palmatic acid) was also found present in the extract. Recent report by the World Health Organization had indicated that palmitic acid increases the risk of developing cardiovascular diseases because of its ability to increase low density lipoprotein levels (Mancini, 2015).

CONCLUSION

Ajumbaise polyherbal is made up of 6 different medicinal plants common in southeast, Nigeria and is heavily enriched with high extract yields and phytocomponents which are of various medicinal values including antioxidants, hypolipidaemia and prevention and management of cardiovascular diseases. More work may be carried out to adequately explore possible medicinal potentials of this polyherbal formulation.

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