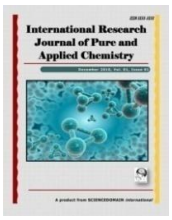

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Chemical Isolation and Characterization of a Popular Detoxifying Herbal Remedy Yoyo Bitters (YYB) Using GC-MS, NMR and FTIR Analysis

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Authors' contributions

Author AAO conceptualized and designed the study, developed the protocol, managed literature search and drafted the manuscript. Authors IOO and ARS finalized the study concept, reviewed the manuscript and contributed to the final draft. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To identify, isolate and characterize the chemical compounds in a popular herbal product consumed in Nigeria.
Study Design: Experimental study.
The use of herbal medicine is a global practice. In Nigeria, a significant percentage of the population rely on herbal medicine to meet their health needs. YYB, a herbal medicine marketed in Nigeria and exported to other parts of the world is used for the alleviation of various health challenges. Previous studies using animal models have tried to elucidate the pharmacological activities of the product, but none has been able to isolate, identify and characterize any active compound in the herbal product. This work sets out to identify the chemical composition of the product towards a better understanding of its pharmacological claims.
GC-MS analysis of various concentrates of the herbal product extracted after column

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chromatography was performed using GC – MSD 5973 Agilent instrument. Structural elucidation of the compounds was performed using NMR and FTIR.
Results showed that the herbal preparation contained both compounds whose pharmacological activities have been established as well as other novel compounds whose full pharmacological activities in humans are yet to be elucidated. Compounds with established pharmacological activities were: 3-n-Hexylthiolane,S,S Dioxide (10.4%); Phenol, 2,4 – bis (1,1 dimethylethyl (5.0%), 4-amino-5,6-dimethoxy-2-cyclohexen-1-ol (13.4%), Furyl hydroxymethyl ketone (1.1%), D-Allose (54.3%),1,6 anhydro-alpha d galactofuranose (42.9%) and Benzene (0.2%).Identified novel compounds that require full elucidation of pharmacological activities were: 1-Fluro Dodecane (18.1%), Hentriancontane (43.4%), Di-N butylphthalate (5.2%), Triacontane,(17.9%), 2-Thiopheneacetic acid oct-3-en-2-yl ester (1.7%), Benzofuran 2,3 dihydro (16.2%) and Thiophene, 2 propyl (29.5%). Others were 1,2,3,4,5 cyclopentanepental (17.2%), 2(1H) naphthalenone, octohydro-8a-hydro-4a-methyl,- cis (1.1%) and 2, 2 dimethyleicosane (3.1%).
The possible biochemical and pharmacological applications of some of the chemical constituents of the herbal preparation were discussed with reference to their therapeutic potentials.

Keywords: Herbal medicine; chemical compounds; pharmacological properties; novel compounds.

1. INTRODUCTION

The World Health Organization (WHO) has defined herbal medicine as: 'any part of the plant in which one or more of its parts can be used for therapeutic purposes or as precursors for the synthesis of useful drugs' [1]. Herbal remedies have been used for several years by people from diverse cultures. Prior to the advent of modern medicine, use of herbal medicine was the only available treatment known to mankind. To date, a significant percentage of people all over the world have relied on herbal medicine to meet their various medical needs. The voracious use of herbal medicine by different people is primarily based on the belief that herbal drugs are safe and may be consumed at any quantity without any side effects. Other reasons are - its accessibility and availability at minimal cost. These factors majorly drive the use of herbal medicine among people living in different countries. Based on the reports of WHO, the use of herbal medicine throughout the world has exceeded the use of conventional therapies by two to three times [2]. Studies conducted in Lagos, South Western Nigeria by Ibrahim et al [3] showed that more than 60% of the surveyed population claimed to have used a herbal preparation either alone or in combination with other herbal medicines.

Plants, herbs, and ethnobotanicals have been used since the early days of humans and are still used throughout the world for health promotion and treatment of diseases. Plants and natural sources form the basis of today's modern medicine and have contributed largely to the commercial drug preparations manufactured today. About 25% of drugs prescribed worldwide are derived from plants [2]. In most developing countries, herbs rather than drugs are often used in health care. For some, herbal medicine is the preferred method of treatment, while for others; herbs are used as adjunct therapy to conventional pharmaceuticals. However, in many developing societies, traditional medicine of which herbal medicine is a core part is the only system of health care available or affordable [4].

YYB, the product used for this study is a herbal product made of extracts of medicinal plants with bitter substances and essential oil. It is a blend of various herbs which contain various secondary constituents such as alkaloids, flavonoids, phenols and polyphenols which are believed to be responsible for scavenging free radicals, The manufacturer of the herbal product under study has carefully formulated the product with a view to reducing free radical damage and removal of harmful toxins from the body, thereby supporting the immune system and the body's ability to resist disease [5].

The herbal formulation has been claimed to be very beneficial to human health. It is believed to improve the circulation of blood, purifies the kidney and reduces the development of kidney stones. Others include: improving digestion, reducing blood pressure, assisting in the elimination of bad cholesterol, preventing the development of diabetes and improving the immune system and memory. Further claims such as pain-free menstruation, reduction in stomach acidity, weight and excess body fat have all been attributed to the product [5,6,7]. In order to elucidate the claims of the manufacturer

on the products, several researchers have conducted a number of biochemical studies using animal models. Oyewo et al. [6], investigated the immune modulatory capabilities of sub-chronic administration of YYB in male wistar rats. The study concluded that YYB administered at a lower dose than the manufacturer's recommended doses produced a much more desired immune modulatory effects. They called for caution in the use of the product at the current recommended manufacturer's dose. Jimmy and Udofia [7] studied the antidiabetic potentials of YYB when compared with known antidiabetic drug, glibenclamide in alloxan induced diabetic rats for the period of 28 days. The study observed that reduction of plasma glucose in the rats was dose and periodic dependent. They concluded that the product could offer some anti diabetic benefits similar to the effect produced by glibenclamide; and that it could be used as alternative drug for the treatment of diabetes. Furthermore, in another study conducted by Ali et al. [8], they investigated the effect of YYB on the morphology of the cerebellum of wistar rats. They deduced from their study that high consumption of YYB for long period of time could have a significant effect on the histology of the cerebellum of wistar rats which might result in cerebella dysfunction. In all of these studies, the researchers have been unable to isolate, characterize and identify any chemical in the product that could be responsible for these observed effects. Premised on these, this study was conducted to isolate, identify and characterize the active compounds present in this popular consumed herbal product. This study is therefore the first attempt made to characterize this widely used herbal medicine.

2. MATERIALS AND METHODS

2.1 Selection of Herbal Products

The herbal product, YYB was purchased from a registered pharmaceutical shop. The product is a combination of several medicinal plants. As an inclusion criterion, the product was ascertained to have been 'registered' with the National Agency for Food, Drug Administration and Control (NAFDAC). The manufactured and expiry dates of the product were inspected and all were confirmed to be within the acceptable time frame. The Manufacturer's seal, inspected to ascertain the authenticity of the product was intact in all the bottles of the syrup purchased for the analysis. In all, about fifteen bottles of the product, each containing a 200 ml of syrup were purchased and used for the required laboratory analysis.

2.2 Extraction

The syrup (10 X 200 ml) was macerated at room temperature in hexane for 24 h. The extract was suctioned and filtered using Whatmann filter paper (size 40). This process was repeated for two more days to ensure complete extraction of the majority of non polar compounds from the syrup. After hexane extraction by maceration, further extraction was performed using ethanol. The extracts were then pooled together and concentrated at 40ºC under reduced pressure using Buchi R-153 Rotavapour. The yield of extract was 24 grams. The resulting residue was then used for subsequent analysis.

2.3 Column Chromatography

The column (3.5 × 60 cm) was prepared with silica gel (60-120 mesh) in n-hexane by wet method and column was left for overnight. 15 mg of ethanolic extract was suspended in water (300 ml) and was poured into the column. The various compounds present in the herbal preparation were then eluted using 500ml combinations of ethyl acetate, methanol, ethanol and water in the ratio of 81: 11: 4: 4 using the principle of liquid/liquid extraction. The entire run time for the separation was 0.75 - 1.0 h. Separation using this process was based on the solubility of various compounds contained in the herbal product in the mobile and stationary phase. In all, three distinct fractions (Fractions 1, 2 and 3) were collected from the herbal product. The initial volume of the fractions eluted was: 75 ml, 125 ml and 100 ml respectively. These fractions were concentrated in vacuum rotary evaporator for 4 - 5 days. After which the final volume obtained for each fraction was 4.5 mL, 5.0 mL and 4.7 mL respectively. These were then used for subsequent analysis.

2.4 GC/MS Analysis on Various Fractions Eluted from Column Chromatography

GC – MS analysis was carried out using GC – MSD 5973 Agilent instrument. Column thickness was 0.25 µm and length was 30 meters with internal diameter of 0.25 mm. Helium was used as carrier gas at a flow rate of 1 ml/min. The column temperature was initially kept at 70ºC and increased to 250ºC at a rate of 10ºC /min. The injector temperature was 250ºC and split

ratio was adjusted at 1:100. The injection volume was 2 µl in ethyl acetate. The detector was Mass Selective Detector. Identification and elucidation of isolated compounds were made using the database of the National Institute of Standards and Technology (NIST) Library. The spectra of the unknown compounds were compared against the standard spectra of known compounds stored in the NIST library. The name, molecular weight and structure of the chemicals contained in the test materials were ascertained. The relative percentage peak area of each compound was calculated by dividing its average peak area with the total area of all compounds present (sum total of all peak areas equal to 100).

2.5 1H NMR Analysis

For this study, 1H NMR, $CDCl_3$ NMR was employed. 1-5 mg equivalent of liquid sample, of fraction 1, 2 and 3 respectively was dissolved in 0.5 - 0.6 mL deuterated solvent and analyzed using Unity AS400 NMR Analyzer. Acquisition Time was between 3-8 Seconds and relaxation (Recycle) Delay was 2-6 Seconds. The frequency was 300-500 MHz and the Nucleus employed was ^1H. Pulse sequence employed was a single pulse, steady pulse was maintained at 8, sweep width was 16 ppm and the solvent used was D2O/$CDCl_3$. The ambient temperature was 25ºC with line broadening at 0.35H_Z. NMR and FTIR analysis were applied to elucidate the structural arrangement of various reactive species nuclei such as hydrogen, carbon, methyl groups and other reactive species present in the isolated chemical compounds.

3. RESULTS AND DISCUSSION

The names of chemicals, molecular formula, molecular weight and peak area of the compounds isolated from various fractions are shown in Tables 1, 2 and 3. The GC-MS spectra of compounds isolated from various fractions showing the retention time and peak area of the various compounds are shown in Figs. 1, 2 and 3. A typical mass spectrum of one of the chemicals obtained after matching with data in the NIST library is shown in Fig. 4. The names and structures of some of the chemicals isolated from the herbal remedy are shown in Fig. 5.

The first pure extracts of the compounds eluted (Fraction 1) was a light brown coloured liquid. On further purification, the fraction did not change its state. A total of six compounds were identified (Table 1) out of which only two compounds were identified to possess biological activities and hence were characterized. The Fourier Transform Infrared Spectroscopy (FTIR) analysis of the hexyl thiolane S,S Dioxide showed spectra 2917,2849,1707 cm^{-1} (carbonyl) 1477, 1472, 1463, 1374 cm^{-1} (sulfonic acid) 1054 (sulfoxide). The GC – MS chromatogram showed two major compounds with retention time (RT) at 8.72 and 10.25 min respectively. The compounds were identified as 3-n-Hexylthiolane,S,S Dioxide ($C_{10}H_{20}O_2S$) (peak 'a') and Phenol, 2,4 – bis (1,1 dimethylethyl ($C_{14}H_{22}O$) (peak 'b'). Peak 'a' showed fragments at m/z 187.3 (M^+-1) , 97, 83,70, 69,57,55 and 55.41 and peak 'b' phenol,2,4-bis (1-1 dimethyl showed fragments at m/z 191 (M), 175, 163, 131, 01, 57 and 41.

3-n-Hexylthiolane, S,S Dioxide is a glycoside. Glycosides are chemical constituents of many natural occurring phytocompounds. The GC-MS analysis of the ethanolic extracts of Habiscus rosa sinensis have also shown the presence of this chemical [9]. The compound has been reported to possess antioxidant and anticancer properties. Glycosides are well known Na^+/K^+-ATPase inhibitors and it is possible that most medical benefits attributed to glycosides could be mediated through this mechanism [9].

Phenol, 2,4 – bis (1,1 dimethylethyl) is an alkyllated phenol and has been classified as a tannin which is one of the major occurring phytocompounds in most medicinal plants. They contain polyphenols which are very reactive with proteins, starches and other macromolecules. Industrially, is used as UV stabilizer and an antioxidant for hydrocarbon based products. It prevents gumming in aviation fuels. It is produced by the reaction of phenol with isobutene catalysed by Aluminium phenoxide. It has a low toxicity with LD50 of 9200 mg/kg [10]. Tannins have been reported to possess other biological benefits such as accelerating blood clotting, reducing blood pressure, decreasing serum lipid level, producing liver necrosis and modulating immunoresponses.[11]. The immune modulatory effects observed from the studies of Oyewo et al. [6] could be attributed to the presence of this chemical.

Compounds such as Hentriancontane, Triacontane , Di-N- butylphthalate (DBP) and 1-Fluro Dodecane have all been reported to possess some chemical activities [12,13,14], but their full pharmacological benefits on humans have not been fully elucidated. Having identified these chemicals in herbal product, the need to

study their pharmacological properties becomes very imperative.

The second pure extracts of the compounds eluted (Fraction 2) was a yellow coloured elute which on further purification did not change its state. A total of eight compounds were identified on GC/MS (Table 2) out of which only three compounds were identified to possess biological activities. The Fourier Transform Infrared Spectroscopy (FTIR) analysis showed 3430 cm^{-1} (hydroxyl), 1645 cm^{-1} (primary amino), 1670 cm^{-1} (ketones), 1472,1463, 1301, 729 and 717 cm^{-1} (methylene chain). After comparison with attached software library data, the compounds were identified as 4-amino-5,6-dimethoxy-2-cyclohexen-1-ol ($C_8H_{15}NO_3$) (peak a) with retention time of 3.41 minutes, Furyl hydroxymethyl ketone ($C_6H_6O_3$)(peak b) with retention time of 5.03 mins and D-Allose ($C_6H_{12}O_6$) (peak c) with retention time of 10.03 mins respectively. Peak 'a' showed fragments at m/z 96(M^+), 85,56.1, 57.1, 55,1. Peak 'b' showed fragments at m/z 126 (M^+), 95.0, 55, 65, 50, 37, Peak 'c' showed fragments at m/z 98 (M^+) , 73, 68,54,47,44.

Table 1. Names of chemicals, molecular formula and peak area isolated from fraction 1

S/N	Retention time (RT) minutes	Name of compound	Molecular formula	Molecular weight	Area of compound	% Peak area
1	6.21	Dodecane 1- fluoro	$C_{12}H_{25}F$	188	621225856	18.1
2	8.72	3-n- Hexylthiolane, S,S Dioxide	$C_{10}H_{20}O_2S$	204	355281600	10.4
3	10.25	Phenol, 2,4 – bis (1,1 dimethylethyl)	$C_{14}H_{22}O$	206	169513632	5.0
4	11.21	Hentriancontane	$C_{31}H_{64}$	436	1488774528	43.4
5	15.16	Di-N butylphthalate	$C_{16}H_{22}O_4$	278	177383072	5.2
6	15.40	Triacontane	$C_{30}H_{64}$	424	612314880	17.9

Table 2. Names of chemicals, molecular formula and peak area isolated from fraction 2

S/N	Retention time (RT) minutes	Name of compound	Molecular formula	Molecular weight	Area of compound	%Peak area
1	3.41	2-cyclohexen-1-ol, 4-amino 5,6 dimethoxy	$C_8H_{15}NO_3$	173	816573952	13.4
2	5.03	Furyl hydroxymethyl ketone	$C_6H_6O_3$	126	69123520	1.1
3	6.06	2- Thiopheneacetic acid, oct-3-en-2-yl ester	$C_{14}H_{20}O_2S$	142	103314432	1.7
4	6.54	Benzofuran 2,3 dihydro-	C_8H_8O	120	987966464	16.2
5	6.66	Thiophene, 2 propyl	$C_7H_{10}S$	126	1803604608	29.5
6	10.03	D-Allose	$C_6H_{12}O_6$	180	1211666328	19.8
7	11.33	1,2,3,4,5 cyclopentanepental	$C_5H_{10}0_5$	150	1053065984	17.2
8	12.51	2(1H) Naphthalenone, octohydro-8a-hydro-4a-methyl,- cis-	$C_{11}H_{18}0_2$	182	68841688	1.1

Table 3. Names of chemicals, molecular formula and peak area isolated from fraction 3

S/N	Retention time (RT) minutes	Name of compound	Molecular formula	Molecular weight	Area of compound	%Peak area
1	3.45	2, 2 dimethyleicosane	$C_{22}H_{46}$	310	103430144	3.1
2	6.73	Benzene (ethylenoxy)	C_8H_8O	120	5545614	0.2
3	10.01	D-Allose	$C_6H_{12}O_6$	180	1843117824	53.8
4	11.20	1,6 anhydro-alpha d galactofuranose	$C_6H_{10}O_5$	162	1471208064	42.9

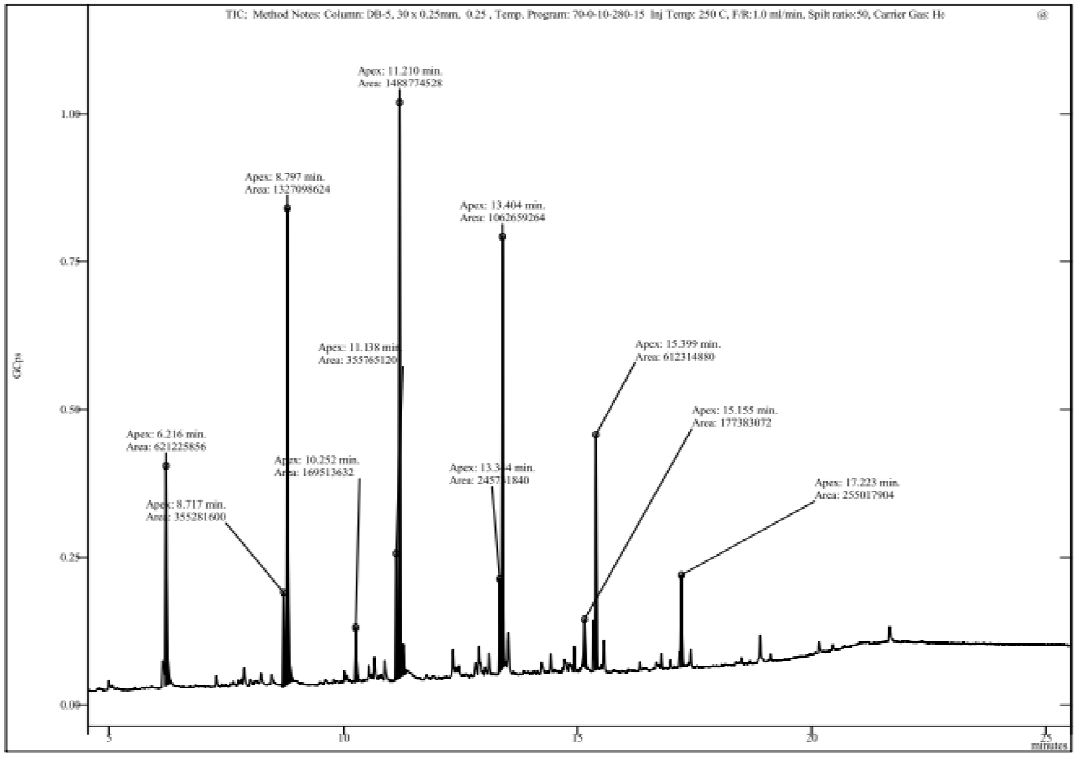

Fig. 1. GC-MS spectra of compounds isolated from fraction 1 of herbal extract showing the retention time and area of various chemicals. The percentage peak area for each chemical is obtained by dividing the individual peak area with the peak area of all the chemicals

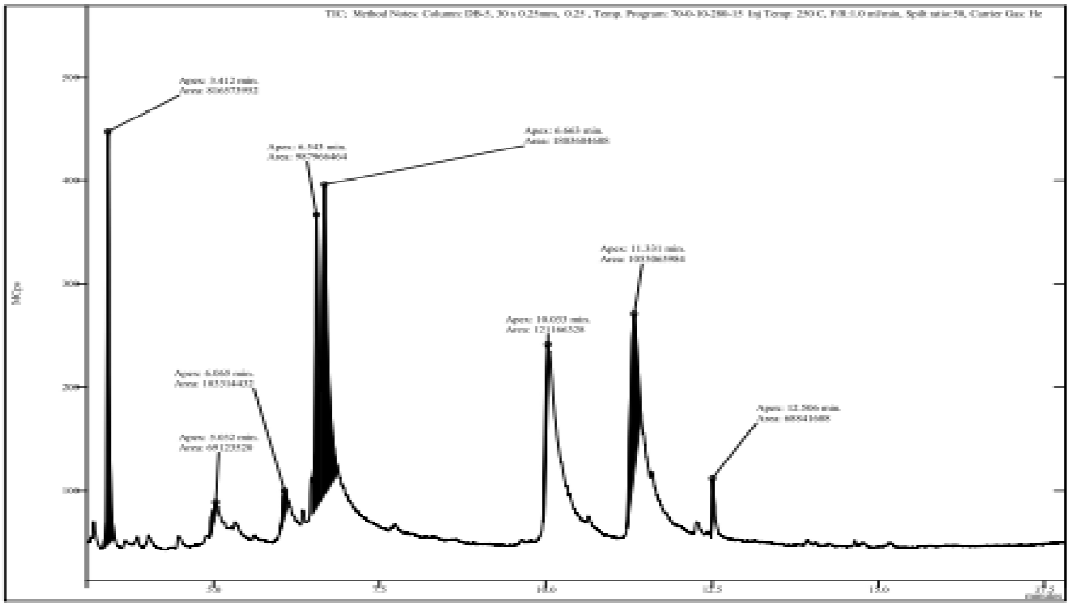

Fig. 2. GC-MS spectra of compounds isolated from fraction 2 of herbal extract

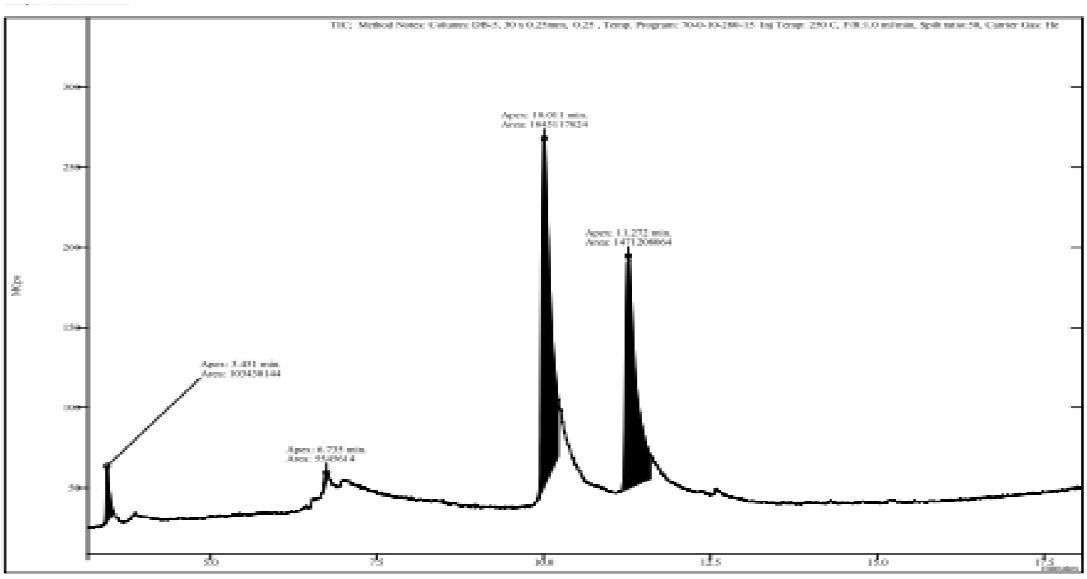

Fig. 3. GC-MS spectra of compounds isolated from fraction 3 of herbal extracts

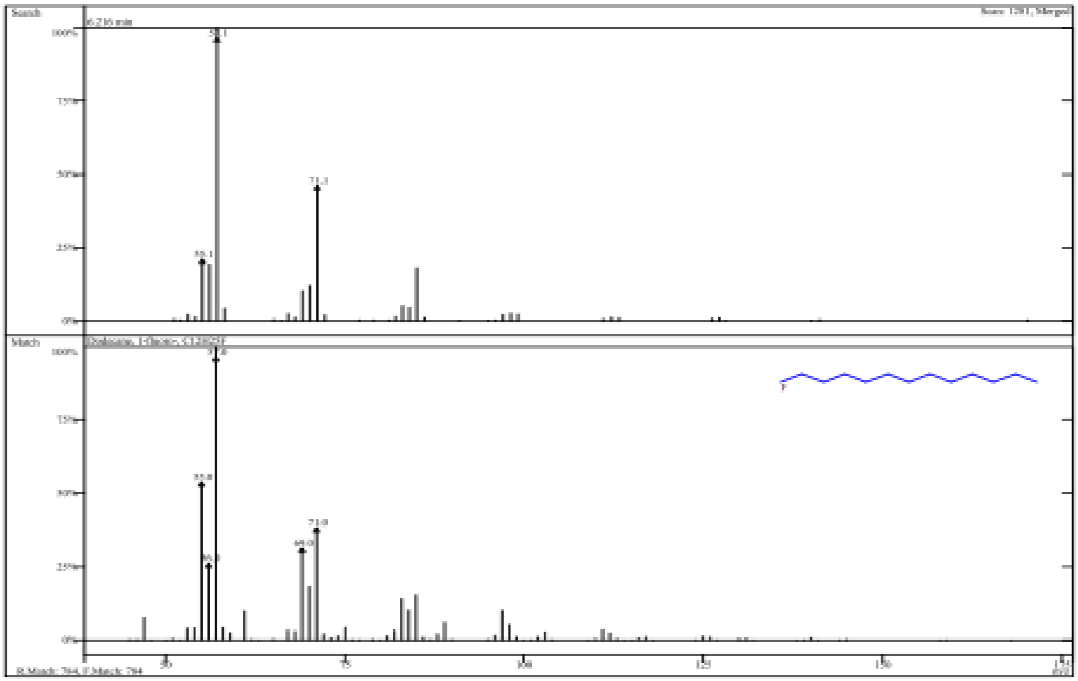

Fig. 4. A typical mass spectrum of one of the chemicals- 1-fluro dodecane obtained after matching with data in the NIST library

4-amino-5,6-dimethoxy-2-cyclohexen-1-ol ($C_8H_{15}NO_3$) is an alkaloid. Alkaloids are common constituents of many phytocompounds. Although the isolation of this chemical has not been previously reported, yet as an alkaloid, it is possible that it could be useful for the synthesis of important drugs. Alkaloids have been identified to possess various pharmacological properties including anagelsics, anesthetic, stimulants, anticancer, antihypertensive, antimalaria, vasodilators, antiasthmatic and antibacterial.

Alkaloids are also responsible for the bitter taste of several phytocompounds.

Furyl hydroxymethyl ketone is a light yellow to brown amorphous compound. It is a glycoside. It is a product of acid catalysed conversion of Hexoses (glucose/fructose) [15]. It occurs naturally in strawberry and has been found in the extract of *Cichorium intybus* root. The toxicological properties of this substance have not been determined, but it may cause respiratory tract infection when consumed in excess [15]. However, recent reports have linked the presence of excess ketones and excessive consumption of ketogenic foods with the body's ability to recover from cancer [16]. The premise being that since cancer cells only require glucose to survive and do not have the in-built mechanism to synthesize the required energy from ketones and ketogenic foods (food rich in protein and fats) unlike the normal body cells, excessive supply of ketones and ketogenic foods starve the cancer cells thus killing the cells. Furthermore, glycosides are well known Na^+/K^+ATPase inhibitors and the beneficial effects attributable to these phytochemicals in the treatment of cancer and inflammatory diseases could be through this mechanism. Nevertheless, the full pharmacological properties of this novel compound are required to be elucidated.

D-allose is the simplest among over 50 simple sugars present in most natural products. Our present study showed that d-allose was one of the products isolated from the herbal remedy. Recent studies with d- allose have shown that it has been implicated in several metabolic pathways where it was identified to play some important and beneficial metabolic roles. In studies using animal models, d-allose was shown to exert immunosuppressive and protective effects against liver damage. It can also inhibit cancer cell proliferation and generation of reactive oxygen species (ROS) in neutrophils [17,18,19].

Recent study comparing the free radicals scavenging properties of some rare sugars, other than d- allose with some other scavengers such as superoxide dismuthase and carotinoids highlighted the relative non- significant difference in their scavenging activities. However, significant inhibition of reactive oxygen species production was detected only when d-allose was added, although the inhibition was found to be dose dependent [20]. Following this observation, it could be concluded that d- allose was strongly implicated in reducing the metabolic consequences secondary to the generation of free radicals and the degree of reduction was dose dependent. Furthermore, the ameliorative effect of d-allose has been observed after liver transplantation and ischemia reperfusion injury of the liver [17,18].

Metabolic processes in the body generate excess free radicals and reactive oxygen species which have been implicated in many disease conditions. In the central nervous system, excessive effects of free radicals results in the excessive secretion of excitory amino acids such as glutamate and aspartate. These amino acids bind to cell membrane of the brain and make it more permeable to ions such as calcium, sodium and water. These excessive ions cause injury to the brain cells, which in the process releases more free radicals, causing cell death. It appears that reactive oxygen species can provoke cell death, either by reacting with cell components, leading to necrosis, or by activating specific targets thus triggering apoptosis [21]. Recent studies with d- allose have shown that it reduces the release of glutamic acids from the brain as a result of excessive generation of free radicals , thereby limiting the deleterious effects of ROS in the brain and other cells [21].

Furthermore, compounds such as: 2-Thiopheneacetic acid, oct-3-en-2-yl ester(glycoside); Benzofuran 2,3 dihydro, Thiophene, 2 propyl, 1,2,3,4,5 cyclopentanepental (tannin) and 2(1H) Naphthalenone, octohydro-8a-hydro-4a-methyl,-cis (flavonoids) have not been previously isolated from herbal products and their biological activities in humans have not been established. There is no doubt that these could be novel compounds which if properly studied, the pharmacological properties could be elucidated, making these chemicals good molecules for the synthesis of new drugs. Furthermore, the level of concentration of these chemicals identified in the herbal products confirms that they are natural occurring phytochemicals and could be useful as drug candidates if pharmacological properties are well elucidated.

The third pure extracts of the compound eluted (Fraction 3) was a yellowish green elute which on further purification did not change its state. A total of four compounds were identified on GC/MS (Table 3) out of which only three compounds were identified to possess biological

activities. The Fourier Transform Infrared Spectroscopy (FTIR) analysis showed 3430 and 1062 cm^{-1} (hydroxyl), 1640, 1463, 1381, 1240, 1023,969,959 cm^{-1} (trans disubstituted double bond), 838 cm^{-1} (trisubstituted double bond). 1HNMR at 400Mz showed the following: 3.55 (1H, m, H-3) and 5.0-5.35 (olefinic protons), 0.68-1.19 (methyl signals), 4.43 (1H, t, j=4Hz, H-3), 5.29 (1H, t, j=4Hz, H-12) and 1.98 (3H,s,$OCOCH_3$).

The three compounds with biological activities that were identified had a retention time (Rt) of 6.73, 10.01 and 11.20 minutes respectively. The compounds were identified as Benzene (ethylenoxy,), D-Allose and 1,6 anhydro-alpha d –galactofuranose respectively. Benzene showed fragments at m/z 120(M^+), 91,94, 77,55.1, 51.2, and 1,6,anhydro-alpha-galactofuranose showed fragments at m/z 98 (M^+),73.0,60, 57.1, 55, 54,after comparison with attached software library data.

Benzene is a colorless, flammable liquid with a sweet odor [25]. It evaporates quickly when exposed to air. Benzene is formed from natural processes, such as volcanoes and forest fires, but most exposure to benzene results from human activities. It is mostly used during industrial activities such as making of chemicals, plastics, lubricants, rubbers, dyes, detergents, drugs, and pesticides. Benzene is also a natural part of crude oil and gasoline, thus motor vehicle exhaust and cigarette smoke contain heavy amounts of benzene. Exposure to benzene can occur at work, in the general environment, and through the use of products containing benzene. Exposure to benzene can also occur from use of water and food contaminated with benzene [22,23,26].

Isolation of benzene from phytochemicals has not been reported, thus the isolation of this chemical from the herbal medicine investigated presented additional knowledge in this field of science. Benzene isolated in this herbal product cloud result from environmental contamination or due to interaction of various substances contained in the herbal product being investigated.

The pathologic effect of benzene has been severally documented [22,23,24,25]. Its identification as one of the active chemicals in this herbal preparation is therefore of great significance. Nevertheless, the concentration of benzene identified in the herbal product was low and might either be as a result of environmental contamination or poor adherence to quality assurance measures.

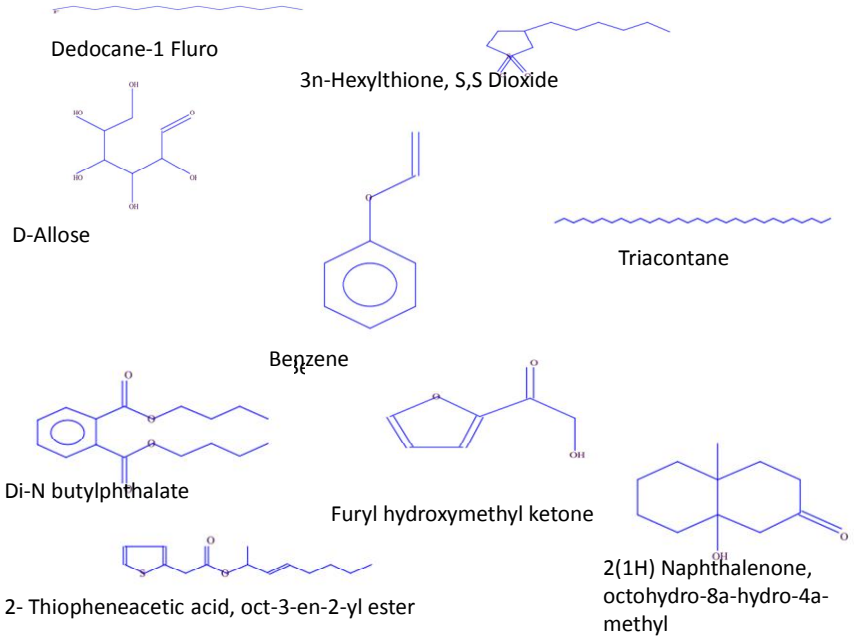

Fig. 5. The names and structures of some of the chemicals isolated from the herbal remedy

The full pharmacological activities of 1,6 anhydro-alpha d –galactofuranose, a glycoside has not been fully evaluated in humans and it is possible that this compound could play a very useful role in modern drug discovery. Furthermore, the isolation of 2, 2 dimethyleicosane, a triterpenoid in herbal medicine and the elucidation of its pharmacological properties have not been documented. Triterpenoids are well known to possess anticancer and anti-inflammatory properties. The elucidation of the pharmacological properties of this compound becomes very imperative.

4. CONCLUSION

The report presented in this study showed that chemicals whose physiological and pharmacological properties have not been investigated were among those isolated from this well consumed herbal product. There is the need to further research into the pharmacological properties of the novel chemicals isolated from this herbal remedy. It is possible that some of the novel compounds isolated if well researched upon, could offer great medical benefit for the treatment of numerous chronic and non communicable diseases challenging the developing and developed world.

5. FURTHER STUDIES

Considering the information generated from this study, there is the need for further studies to investigate the full pharmacological properties of all the novel compounds isolated from this herbal product.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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