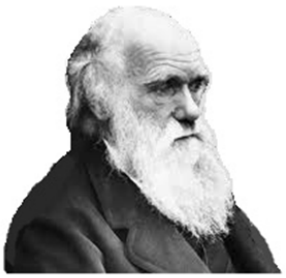
Available online at www.worldnewsnaturalsciences.com



World News of Natural Sciences

An International Scientific Journal

WNOFNS 37 (2021) 1-17 EISSN 2543-5426

Phytochemical and *in vitro* antioxidant assessment of Yoyo bitters

Paula-Peace O. James-Okoro[1], Franklyn N. Iheagwam[1], Mariam I. Sholeye[1], Itoroobong A. Umoren[1], Babatunde O. Adetuyi[2], Adebanke E. Ogundipe[1], Adefoyeke A. Braimah[3], Tobi S. Adekunbi[1], Oluseyi E. Ogunlana[4], Olubanke O. Ogunlana[1,*]

[1]Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria

[2]Department of Natural Sciences, Biochemistry Unit, Precious Cornerstone University, Ibadan, Oyo State, Nigeria

[3]Independent Researcher, 4 Ilupeju Street, Anisere, Sango, Ota, Ogun State, Nigeria

[4]Department of Biological Sciences, Crawford University, Igbesa, Ogun State, Nigeria

*Email-address: banke.ogunlana@covenantuniversity.edu.ng

ABSTRACT

In this paper, herbal bitters are widely used due to their numerous acclaimed health benefits in many Nigerian homes; however, many have not been subjected to scientific scrutiny. The aim of this study was to determine the phytochemical composition and antioxidant capacity of a non-alcoholic polyherbal formulation, Yoyo bitters, towards validating its broad pharmacological claims. The phytochemical components of Yoyo bitters were ascertained by phytochemical screening assays and gas chromatography-mass spectrometry (GC-MS). The antioxidant activity was investigated *in vitro* using 2,2-diphenyl-1-picryhydrazyl (DPPH) radical, hydrogen peroxide (H_2O_2) scavenging activity, total antioxidant capacity (TAC) and ferric reducing antioxidant power (FRAP) assays. Qualitative phytochemical analysis of Yoyo bitters showed the presence of saponins, tannins, flavonoids, terpenoids, cardiac glycosides and anthocyanins. The total phenols, flavonoids, flavanols, tannins and carotenoids content were 14.741 ±0.64 mg GAE/ml, 0.152 ±0.01 mg RE/ml, 0.437 ±0.02 mg RE/ml, 0.368 ±0.04 mg TAE/ml and 0.016 ±0.00 mg CAE/ml respectively. GC-MS chromatogram revealed the presence of forty-three (43) phytochemical compounds with D-allose (41.81%), 1,6-anhydro-beta-D-glucofuranose (24.15%), 5-hydroxymethylfurfural (8.02%) and Z-6-pentadecen-1-ol acetate (3.50%) as the most abundant constituents. Yoyo bitters demonstrated effective antioxidant activity against DPPH

(Received 02 April 2021; Accepted 21 April 2021; Date of Publication 22 April 2021)

and H_2O_2 with IC_{50} values of 0.492 mg/ml and 0.629 mg/ml respectively compared to ascorbic acid of 0.161 mg/ml and 0.130 mg/ml respectively. Total antioxidant capacity and ferric reducing antioxidant power of Yoyo bitters were 0.432 mg AAE/ml and 2.236 mg AAE/ml respectively. This study validates the antioxidant capacity of Yoyo bitters and provides chemical basis for its acclaimed pharmacological actions.

***Keywords*:** Yoyo herbal bitters, GCMS, phytochemical analysis, antioxidant assay, in vitro

1. INTRODUCTION

Herbal medicine is the oldest and remains the most widely used medicine system in the world till date. Herbal medicine is any plant or its parts, with little to no chemical processing, that can be used for therapeutic purposes or as precursors for the production of beneficial drugs [1, 2]. In most developing countries, plants are widely used for treating numerous diseases, as they possess rich medicinal properties. Herbal remedies are frequently regarded as a balanced and moderate solution to disease treatment [3] and many individuals use as them as their primary healthcare solution. The most important explanations for choosing herbal medication are that it is relatively cheap, readily available, dispels fears regarding the negative consequences of pharmaceutical (synthetic) medication, addresses a need for more customized healthcare, and provides for general exposure to knowledge regarding health. Medicinal plants may be processed into herbal bitters. Bitters are beverages, mostly alcoholic, infused with herbal orbs which give it a bitter flavour [4].

Often, they are made from herbs and roots extracts of medicinal plants and spices. In Nigeria, bitters are being sold as a "cure-all" patent medication. Herbal bitters are widely used because of their health benefits and in many Nigerian homes they have become routine medicines. Ethnomedical, herbal medicine was confirmed for the prevention, diagnosis, control and cure of a lot of diseases. The therapeutic strength of these plants is told lie in the phytochemicals present in them [5]. Yoyo bitters is an oral non-alcoholic polyherbal liquid preparation which upon launching became widely marketed and used in Nigeria by the general public. It is a medicine derived from plants, classified as herbal bitters. Yoyo bitters was launched into the market by Abllat Company Nigeria Limited in 2003. The contents of yoyo bitters as published by the producers include *Aloe vera, Acinos arvensis, Citrus aurantifolia, Chenopodium murale, Cinnamomum aromaticum*.

The bitters also contain water, soluble vitamins (e.g., vitamins B1, B2, B3, B6, B12) and minerals (copper, zinc, iron). These components have diverse medicinal purposes. Some of the acclaimed health benefits of Yoyo bitters as stated on the leaflet include prevention and management of diabetes and cancer, relief for ulcer, antioxidant generation, detoxification, blood pressure regulation, anti-microbial, immunostimulant, antimalarial, relief for stomach discomfort, treatment of malnourishment, anti-hypertensive, hypocholesterolemic, weight loss, skin rejuvenation, among other numerous medicinal benefits. The oral polyherbal formulation, Yoyo bitters is a common sight in Nigerian homes. It has been estimated that more than 80% of the African populace depend on herbal medicine as their primary healthcare [1]. There has been a drastic increase in the occurrence of metabolic disorders among the human race. Oxidative stress has been associated with many of these metabolic disorders [6]. The claimed effectiveness of Yoyo bitters has culminated in tremendous popularity for the herbal brand and

many individuals indiscriminately use herbal bitters as the primary cure for metabolic disorders, as its acclaimed medicinal properties can help with alleviating rising metabolic disorders. However, there is little scientific data to support or dispute medicinal claims attributed to the bitters.

The increase in the usage of herbal medicines as a primary healthcare remedy, along with weak regulations to the general access of such products, underlies research initiatives to assess their physiological impact. To this end, the objective of this study is to investigate the phytochemical constituents and antioxidant capacity of Yoyo bitters to better understand its acclaimed pharmacological actions.

2. MATERIALS AND METHODS

Material

Yoyo bitters were purchased from a reputable supermarket in Ota, Ogun State, Nigeria in February, 2020. The bitters were bought as liquid formulations and stored at room temperature throughout the period of the experiment.

2. 1. Qualitative Phytochemical Analysis

Phytochemical assays were performed on yoyo bitters to ascertain the constituent phytochemicals using standard methods as defined [7, 8]. The presence of tannns saponins, flavonoids, alkaloids, anthocyanin, betacyanin, quinones, glycosides, cardiac glycosides, terpenoids, triterpenoids, phenols, coumarins, steroids and carbohydrates were screened in the Yoyo bitters.

2. 2. Quantitative Phytochemical Analysis

Phytochemicals were quantified following the procedure outlined [9]. Total phenols, tannins, carotenoids, flavonoids and flavonols were determined by spectrophotometric methods and the results were expressed as mg gallic acid equivalents per ml (mg GAE/ml), mg tannic acid equivalents per ml (mg TAE/ml), mg CAE/ml and mg rutin equivalent per ml (mg RE/ml) of Yoyo bitters respectively.

2. 3. *In Vitro* Determination of Antioxidant Activity

In this study, four complementary assays were used to evaluate the antioxidant capacity of yoyo bitters: DPPH and H_2O_2 radical-scavenging activity, TAC and FRAP assays. The antioxidant activity was evaluated using the method outlined [9].

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Free Radical Scavenging Assay

The antioxidant capacity of Yoyo herbal bitters was determined in relation to hydrogen donating activity or ability to scavenge radicals using the stable radical, DPPH. 0.5 ml aliquots of sample and standard in various concentrations was treated with 0.5 ml of DPPH. 0.5 ml of DPPH and 0.5 ml dimethyl sulfoxide (DMSO) were put into control test tubes. Incubation was the done at 37 °C for 30 min in the dark. Absorbance was taken at 517 nm with a spectrophotometer UV-Visible. The standard used was ascorbic acid. The absorbance was compared to the calibration curve for ascorbic acid. The results were presented as mg ascorbic

acid equivalent per ml (mg AAE/ml) of herbal bitters. The percentage scavenging activity/inhibition of DPPH radical was determined using the following equation:

$$Percentage\ DPPH\ inhibition\ = \frac{(Absorbance\ of control - Absorbance\ of sample)}{Absorbance\ of control} \times 100\%$$

where absorbance of control = total radical activity without inhibitor and absorbance of test sample = activity in the presence of test compounds.

Hydrogen Peroxide (H_2O_2) Scavenging Activity

The H_2O_2 radical scavenging ability of Yoyo bitters was ascertained following the method outlined [9] with small alterations. 100 µL of Yoyo bitters was incubated with 0.6 mL of H_2O_2 (40mM in a phosphate buffer, 0.1M pH 7.4) for 10 mins in the dark. A negative control was created parallel to the whole reagent excluding the bitters or standard. The absorbance of H_2O_2 was determined at 230 nm against a blank solution comprising a phosphate buffer. The percentage inhibition of hydrogen peroxide was evaluated with the same formula as DPPH inhibition.

Total Antioxidant Capacity (TAC)

TAC assay of Yoyo bitters was performed. 0.3 mL of sample was mixed with 3 mL of reagent solution (0.6 M sulphuric acid, 28 mm sodium phosphate and 4 mM ammonium molybdate). The mixture was sealed and incubated at 95 °C for 90 min. The mixture was allowed to cool to room temperature after which the absorbance was taken at 695 nm against blank solution (0.3 mL methanol). The standard used was ascorbic acid.

Ferric Reducing Antioxidant Power (FRAP)

FRAP test was carried out on Yoyo bitters. 2.5 mL of phosphate buffer (0.2m, pH 6.6) and 2.5 mL of potassium ferricyanide (1%) was combined with 1mL of sample. The reaction mixture was incubated for 20 minutes at 50 °C. 2.5 mL of trichloroaetic acid was then added, after which the mixture was centrifuged for 10 mins. A 2.5 mL aliquot was combined with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1%). The absorbance of all solutions was taken at 700 nm and expressed as mg ascorbic acid equivalent per ml of sample (mg AAE/ml sample).

2. 4. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Yoyo Bitters

GC-MS was used to properly characterize Yoyo bitters. The GC-MS analysis was performed with the aid of GC – MSD 5975 Agilent instrument. Column length, thickness and internal diameter were 30 meters, 0.25 µm and 0.32 mm respectively. The carrier gas used was helium at a flow rate of 10 mL/min. The temperature of the column was first maintained at 80 ºC before it was increased, at a rate of 10 ºC/min, to 290 ºC.The temperature of the injector was 250 ºC and the split ratio was modified at 1: 100.The injection volume was 2 µL in ethyl acetate and Mass Selective Detector was used as the detector. The relative percentage peak area of each phytocompound was determined by dividing the average peak area with the total area of all the present compounds. The detected peaks were interpreted by comparison with data from the National Institute of Standards and Technology (NIST) to determine the names and molecular weights of the test sample components.

3. RESULTS

Phytochemical Screening

Table 1 shows the qualitative phytochemical analysis of Yoyo bitters. Yoyo bitters contains saponins, flavonoids, anthocyanins, cardiac glycosides and terpenoids.

Table 1. Qualitative phytochemical analysis of Yoyo bitters.

Phytochemicals	Yoyo Bitters
Tannins	-ve
Saponins	+ve
Flavonoids	+ve
Alkaloids	-ve
Beta-cyanins	-ve
Anthocyanins	+ve
Phenols	-ve
Quinones	-ve
Glycosides	-ve
Cardiac glycoside	+ve
Terpenoid	+ve
Triterpenoid	-ve
Coumarins	-ve
Carbohydrates	-ve
Steroids	-ve

+ve = Present; -ve = Absent.

Table 2 shows the quantitative phytochemical assessment of Yoyo bitters. Upon quantitative analysis of the bitters, the total phenol, flavonoid, flavanol, tannin and carotenoid contents in yoyo biters were found to be 14.741 ± 0.64 mg GAE/ml, 0.152± 0.01 mg RE/ml, 0.437± 0.02 mg RE/ml 0.368 ± 0.04 mg TAE/ml and 0.054 mg CAE/ml respectively.

The quantitative phytochemical analysis of yoyo bitters indicates phenols as the most abundant constituent and β-carotene the least among the phytochemicals determined.

Table 2. Quantitative phytochemical analysis of Yoyo bitters.

Phytochemicals	Yoyo Bitters
Total Phenols (mg GAE/ml)	14.741 ±0.64
Total Flavonoids (mg RE/ml)	0.152 ±0.01
Total Flavonol (mg RE/ml)	0.437 ±0.02
Total Tannin (mg TAE/ml)	0.368 ±0.04
Carotenoids (mg CAE/ml)	0.054 ±0.00

Values represent mean ± SD of triplicate determinations.

In Vitro Antioxidant Activity

Table 3 shows the IC_{50} values of Yoyo bitters and ascorbic acids. Yoyo bitters has the ability to scavenge the DPPH and H_2O_2 radical with IC_{50} values of 0.629 mg/ml and 0.492 mg/ml respectively compared to 0.161 mg/ml and 0.131mg/ml for ascorbic acid.

Table 3. IC50 of Yoyo bitters.

	IC50 (mg/ml) by DPPH scavenging ability	IC50 (mg/ml) by H_2O_2 scavenging ability
Yoyo Bitters	0.629	0.492
Ascorbic Acid (Standard)	0.161	0.131

Values represent mean of triplicate determinations.

Figures 1 & 2 show the graph of DPPH and H_2O_2 antioxidant assay of Yoyo bitters. The graph shows a dose-dependent increase in the scavenging activity of DPPH and H_2O_2 radical for Yoyo bitters and ascorbic acid.

Figures 3 & 4 shows the total antioxidant capacity and the reducing power of Yoyo bitters. A dose-dependent improvement was noticed for the two assays with 0.432 ± 0.003 mgAAE/ml as the highest antioxidant capacity and 2.236 ± 0.03 mgAAE/ml as the highest reducing power recorded.

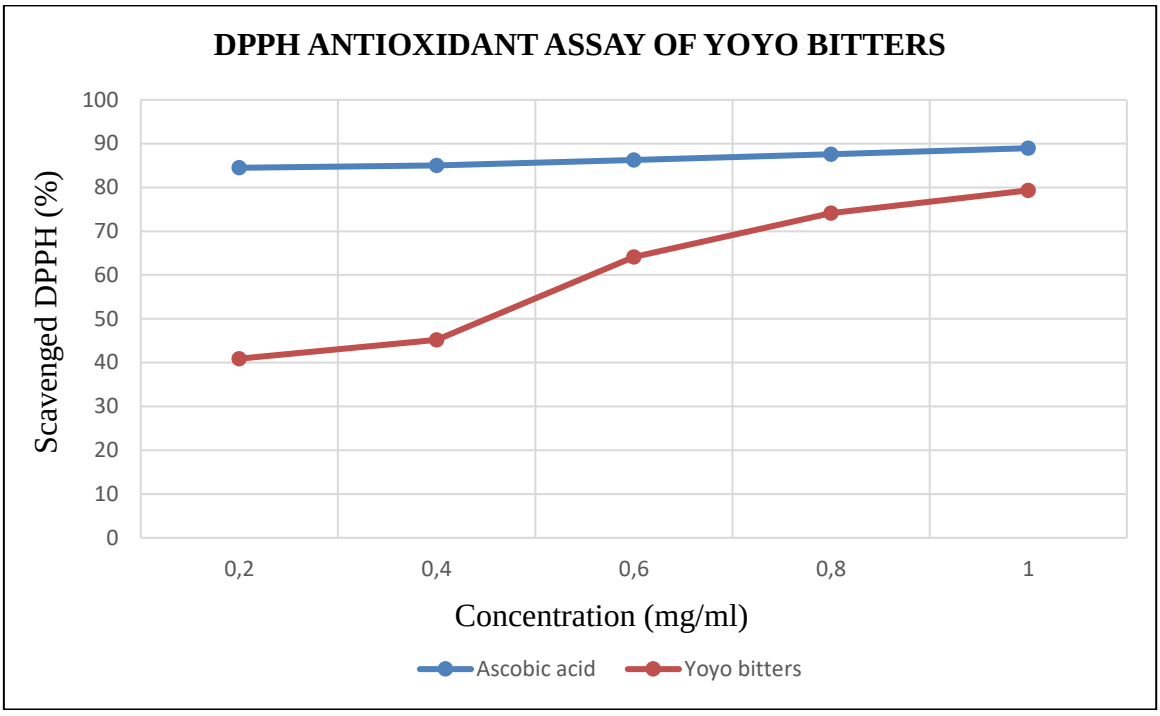


Figure 1. DPPH radical scavenging activity of Yoyo bitters and ascorbic acid. Percentage inhibition of DPPH vs concentration plot of Yoyo bitters and ascorbic acid.

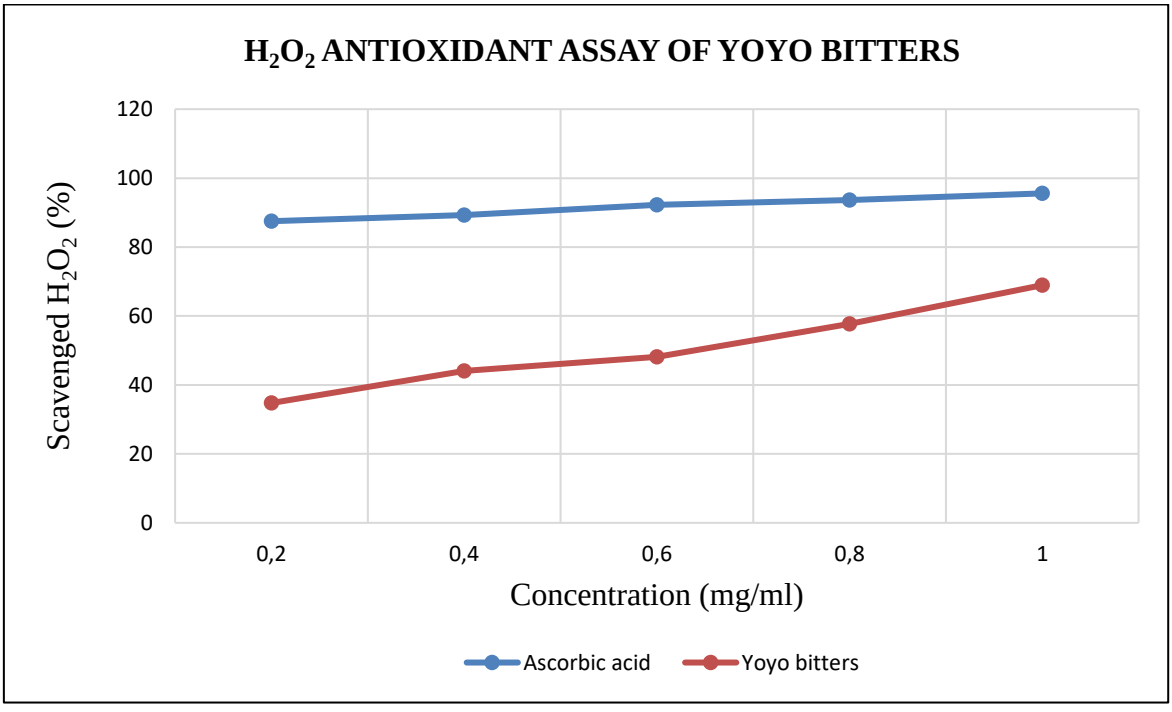


Figure 2. H_2O_2 scavenging activity of Yoyo bitters and ascorbic acid. Percentage inhibition of H_2O_2 vs concentration plot of ascorbic acid and ascorbic acid.

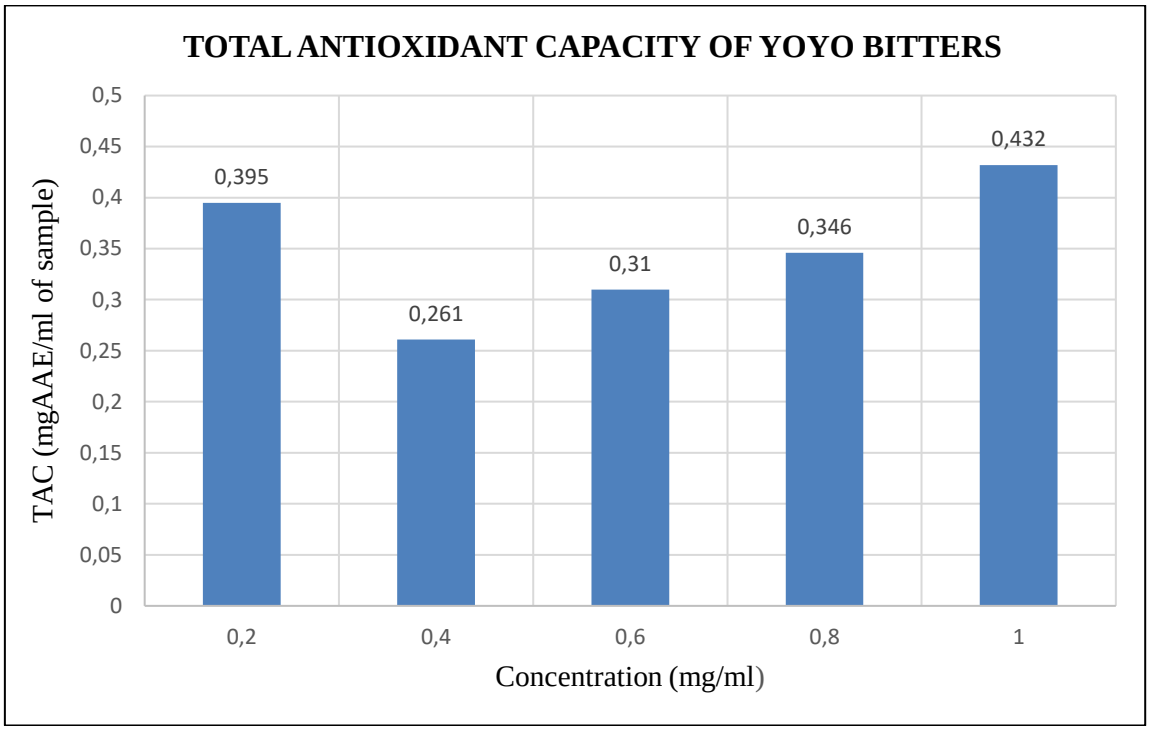


Figure 3. Total antioxidant capacity of Yoyo bitters.

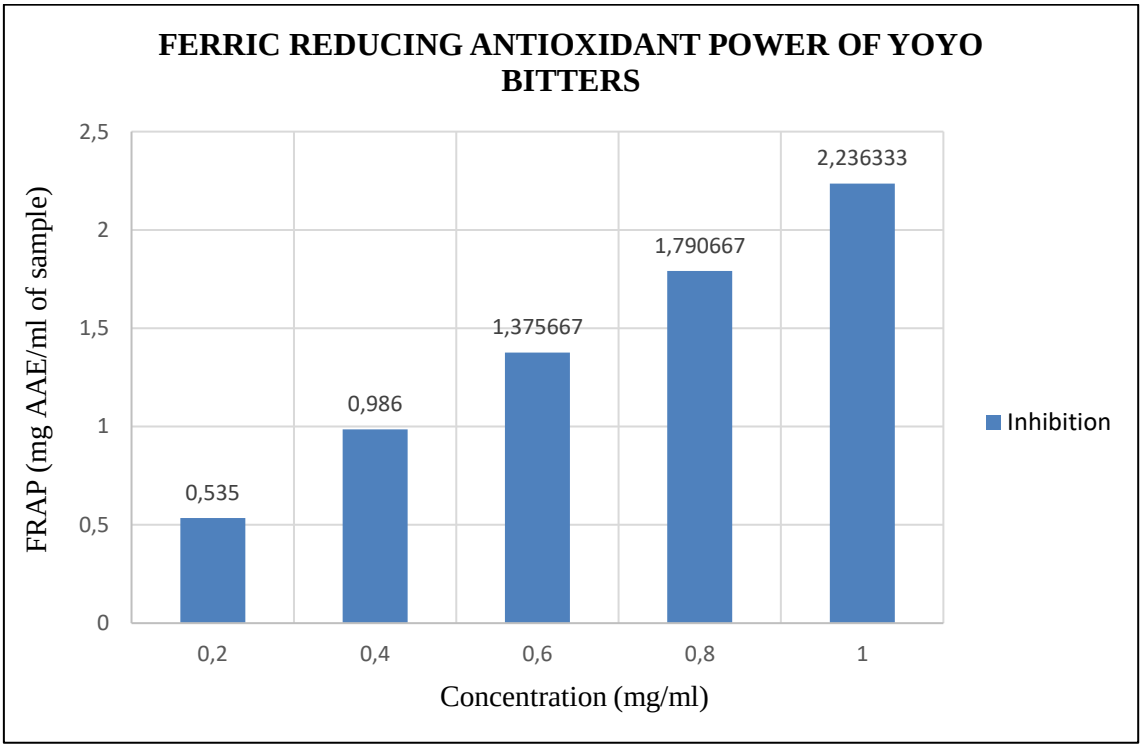


Figure 4. Ferric reducing antioxidant power of Yoyo Bitters.

The results of all four assays indicates that Yoyo bitters contain antioxidant compounds which are good electron donors and can adequately scavenge free radicals.

3. 1. GC-MS Profiling

GC-MS chromatograms of Yoyo bitters is shown in Figure 5. The peak area, retention time and molecular formula were used for the confirmation of identified phytochemical compounds. The GC-MS chromatogram identified forty-three (43) compounds. The list of identified active compounds, peak area percentages, retention times and molecular formulas are presented in Table 4. The most abundant compound is D-Allose (41.81%) and the least abundant compounds are 9-methyl-Z-10-pentadecen-1-ol, 3(2H)-Furanone, dihydro-5-isopropyl and alpha-D-glucopyranoside, O-alpha-D-glucopyranosyl-beta-D-frucofuranosyl (0.08%). Some of the phytocompounds identified in yoyo bitters have reported biological activities. The association of some of the compounds identified with the reported pharmacological properties is illustrated in Table 5.

Table 4. Phytochemical compounds identified for different peaks in Yoyo Bitters.

Peak	Retention time	Area %	Height %	Name
1	4.975	0.14	0.67	1,2-Cyclopentanediol, trans-
2	5.099	0.47	2.17	2,2-Dimethyl-1,3-butanediol
3	5.143	0.73	1.89	Azacyclotridecan-2-one
4	5.242	0.23	1.45	2(5H)-Furanone, 4-hydroxy-3,5-dimethyl-
5	5.294	0.38	1.37	1,2-Epoxynonane
6	5.361	0.82	2.57	Cycloheptane
7	5.472	0.42	1.61	1-Pentanol-2,3-dimethyl-
8	5.658	0.11	0.43	Z-3-Methyl-2-hexenoic acid
9	5.775	2.09	4.29	2-Hydroxy-gamma-butyrolactone
10	5.916	0.67	1.50	1,6-Anhydro-2,4-dideoxy-beta-D-ribo-hex
11	6.077	0.39	1.12	Isosorbide Dinitrate
12	6.242	0.36	1.13	Acetic acid, 2-(1-buten-3-yl)-2-nitro-ethyl
13	6.294	0.58	1.58	1H-Azepin-1-amine, hexahydro-
14	6.326	0.08	0.61	9-Methyl-Z-10-pentadecen-1-ol
15	6.399	0.40	1.38	2-Furancarboxylic acid, 4-pentadecyl ester
16	6.464	0.20	0.96	1H-Azepin-1-amine, hexahydro-
17	6.568	0.55	1.61	2(3H)-Furanone, dihydro-4,4-dimethyl-

18	6.642	0.46	2.42	1H-Azepin-1-amine, hexahydro-
19	6.704	0.64	1.95	Propene, 3-tert-butoxy-2-(methoxymethyl)-
20	6.782	0.51	1.42	2-Furancarboxylic acid, 2,2-dimethylpropyl
21	6.977	0.48	1.62	2(3H)-Furanone, 5-butyldihydro-4-methyl-
22	7.052	1.24	3.13	1H-Azepin-1-amine, hexahydro-
23	7.135	0.48	1.39	Carbonic acid, allyl nonyl ester
24	7.235	0.17	0.37	1,2-Diazabicyclo [2.2.2] octan-3-one, 2-hydr
25	7.485	0.37	1.17	9-Oxabicyclo [3.3.1] nonan-2-one, 5-hydroxy
26	7.540	0.08	0.77	alpha-D-glucopyranoside, O-alpha-D-glucopyranosyl-beta-D-frucofuranosyl
27	7.596	0.09	0.41	Cyclopentane undecanoic acid
28	7.662	0.48	1.87	5-Hydroxymethylfurfural
29	7.739	0.08	0.53	3(2H)-Furanone, dihydro-5-isopropyl
30	8.024	0.72	2.78	1,4:3,6-Dianhydro-alpha-d-glucopyranose
31	8.076	1.69	3.40	3-Propylglutaric acid
32	8.124	2.59	3.78	Cyclohexanone, 2-(hydroxymethyl)-
33	8.196	8.02	14.52	5-hydroxymethylfurfural
34	11.032	0.23	0.32	alpha-D-glucopyranoside, O-alpha-D-glucopyranosyl-beta-D-frucofuranosyl
35	12.417	41.81	11.61	D-Allose
36	13.805	24.15	6.81	1,6-Anhydro-beta-D-glucofuranose
37	17.666	0.39	2.35	9,12-Octadecadienoic acid, methyl ester
38	17.732	0.45	3.01	6-Octadecenoic acid, methyl ester, (Z)-
39	18.597	0.41	0.48	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy] methyl] ethyl ester
40	19.299	0.10	0.54	8-Methyl-6-nonenamide
41	19.848	1.39	0.95	2-Myristynoyl-glycinamide
42	20.187	0.87	1.07	d-Mannitol, 1-decylsulfonyl-
43	21.304	3.50	5.02	Z-6-Pentadecen-1-ol acetate
		100	100	

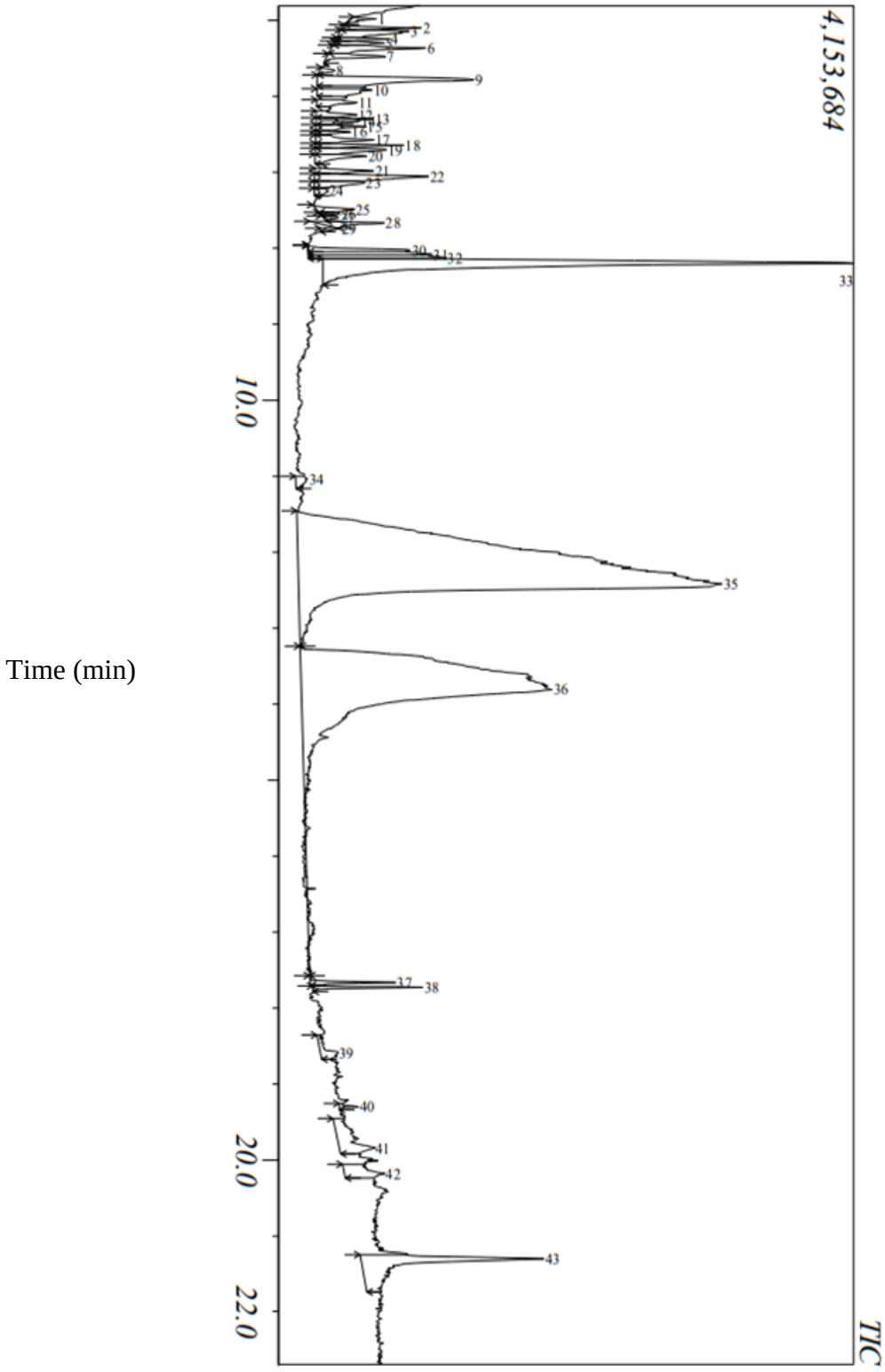


Figure 5. GC-MS spectral chromatogram of Yoyo bitters.

From Figure 5, the OX axis represents the retention time (RT) in mins-the amount of time taken for the analytes to pass through the column and reach the mass spectrometer detector while the OY axis represents the concentration/intensity (peak area) of each compound – a reflection of the amount of a specific analyte that's present. NB: Each peak represents an individual compound that was separated from a sample mixture.

Table 5. Biological Activities of some identified phytocompounds in Yoyo bitters [10-20].

Compound	Biological Activity
D-Allose	Antioxidant, immunosuppressive, hepatoprotective
5-Hydroxymethylfurfural	Antioxidant, antiproliferative
2-Furancarboxylic acid	Bactericide, fungicide
9,12-Octadecanoic acid	Anti-inflammatory, antioxidant, antimicrobial, anticancer, hepatoprotective, antihistaminic hypocholesterolemic, anti-acne, nematicide, anti-arthritic, anti-coronary, anti-eczemic
2-Myristynoyl-glycinamide	Antimicrobial
Cyclopentane undecanoic acid	Antimicrobial
Octadecanoic acid, methyl ester	Antioxidant
9,12,15-Octadecatrienoic acid	Anti-inflammatory, cancer preventive, hepatoprotective, antioxidant, hypocholesterolemic
1,6-Anhydro-β-D-glucofuranose	Blood coagulant, anti-human immunodeficiency virus (HIV)
Isosorbide dinitrate	Vasodilator, hypotensive
2-hydroxygamma-butyrolactone	Epigoitrin from 4-hydroxy-γ-butyrolactone presents antithyroid and antivirus activity

4. DISCUSSION

Yoyo bitters is widely used for the management and treatment of several ailments. Many pharmacological claims have been attributed to the bitters [21]. To validate these claims, it is

necessary to determine the compositional data of Yoyo bitters. The qualitative and quantitative assessment of a medicinal plant's phytochemical components is seen as a significant phase in its research [22]. These results of phytochemical assessment of Yoyo bitters in this study are consistent with the findings [23]. The identified and quantified phytochemicals have reported medicinal properties and may synergistically impact the different pharmacological claims attributed to Yoyo bitters. Phenolics are widely distributed among plants and have reported wide spectrum of biological activities including potent free radical scavenging ability. Phenols and phenolic compounds have reported antioxidant, anticancer, antimicrobial, anti-inflammatory, anti-clotting activities [24]. They prevent a number of diseases because they are able to eliminate free radicals. Flavonoids are the prominent group of phenols. They exhibit numerous chemical and pharmacological activities.

Flavonoids have antimicrobial, cytotoxic, anti-inflammatory, hormone modulator, anti-allergic, immune enhancer, diuretic, anti-spasmodic, hypocholesterolemic, hepatoprotective, vascular properties [25]. Flavonoids have been reported to have strong antioxidant properties, with the ability to scavenge a wide range of free radicals, inhibit lipid peroxidation and are used for therapeutic purposes. Tannins are polyphenolic phytochemicals with reported pharmacological benefits including anti-inflammatory, anti-oxidant, anti-tumor, anti-infective, blood clotting, hypolipidemic, immunomodulatory and antimicrobial [26]. Phenolics are the most common antioxidants present in human diet. The phenol group constituent of all polyphenolics imparts the antioxidant property on plants containing them.

The presence of these phenolic compounds including flavonoids, tannins and phenols may be associated with the antioxidant properties of Yoyo bitters. Saponins have reported wound healing, hypocholesterolemic, hemolytic, antimicrobial, anti-inflammatory, immunostimulant and blood clotting properties [27]. Terpenoids are regarded as having antimicrobial, diuretic, anti-cancer, anti-ulcer, anti-malarial, hepaticidal and anti-inflammatory activities [28]. Cardiac glycosides have cardiotonic, anticancer, antineoplastic activities [29]. They are used for treating heart diseases [30].

This shows that the pharmacological actions of Yoyo Bitters can be credited to the present phytochemicals in the herbal product. Gas Chromatography-Mass Spectrometry (GC-MS) is widely utilised to identify the chemical constituents of medicinal plants [18]. In this study, GC-MS analysis was used to quantify and identify the phytoconstituents of Yoyo bitters. This analysis can help to better understand the pharmacological value of Yoyo bitters. GC-MS analysis of the bitters revealed 43 components with the major components observed in high percentages being D-allose (41.81%), 1,6-anhydro-beta-D-glucofuranose (24.15%), 5-hydroxymethylfurfural (8.02%) and Z-6-pentadecen-1-ol acetate (3.50%) (Table 4).

GC-MS analysis disclosed the presence of medicinally valued phytocomponents and their reported biological activities validates some of the medicinal claims of Yoyo bitters. The reported biological activities of some of the identified phytochemicals include antioxidant, antimicrobial, anticancer, anti-inflammatory, hepatoprotective, hypocholesterolemic, antihistaminic and so on. D-allose is the most prominent phytoconstituent and it has been reported to have potent antioxidant activity.

D-allose has been reported to perform essential metabolic and advantageous roles in several metabolic pathways. Studies have shown that D-allose has the capacity to adequately scavenge free radicals, suppress the immune system, protect the liver from damage as well as inhibit cancer cells proliferation [10-12]. 5-hydroxymethylfurfural has also been shown to have antioxidant properties as well as anticancer activities [13].

The compounds identified by phytochemical screening and GC-MS analysis and their reported biological activities may explain the various pharmacological attributes of Yoyo bitters, especially the antioxidant properties. Antioxidants eliminate free radicals thus, prevent oxidative damage and propagation of oxidation chain reaction caused by free radicals [31]. Thus, antioxidants are very essential in preventing chronic and degenerative diseases. The assays used to assess the antioxidant capacity of Yoyo bitters are based on different mechanisms: DPPH and H_2O_2 assays are based on electron and hydrogen atom transfer, while FRAP is based on electron transfer and reduction of ferric ion to ferrous ion [32].

All assays used in this study demonstrate that Yoyo bitters has potent antiradical and antioxidant activity. The IC_{50} values of Yoyo bitters demonstrates it has good free radical scavenging activity which corresponds with the findings of Anionye and Onyeneke [23]. Yoyo bitters also demonstrated strong reducing power, showing that the antioxidant compounds present in yoyo bitters are good electron donors. Yoyo bitters can thus be said to have high antioxidant capacity. It has been reported that phytochemical constituents present in herbal medicines show significant antioxidant activity and prevent a number of diseases through this activity [33].

Therefore, these compounds may be responsible for the antioxidant capacity of herbal remedies. Antioxidant effect of Yoyo bitters is imputable to the presence of phytochemicals like phenolics, flavonoids, tannins, D-allose which are present in significant quantity. These phytochemicals could thus play a dominant role in the clinical properties of yoyo bitters. Most of the pharmacological claims of Yoyo bitters may therefore be attributed to the presence of several chemical constituents with reported biological activities.

5. CONCLUSION

In this paper, the study justifies the consumption of Yoyo bitters for preventive and therapeutic purposes. The results of this study, with references to some of the reported activities of the chemical components of Yoyo bitters, provide scientific evidence for the acclaimed medicinal benefits. The results of the assays of this study shows that Yoyo bitters is a potent source of natural antioxidants. Thus, the acclaimed therapeutic effects of Yoyo bitter are justified. However, moderate consumption is advised due to the presence of some potentially toxic phytochemicals like tannins and saponins.

ACKNOWLEDGEMENT

The authors thank the Department of Biochemistry, Covenant University, Ota, Ogun State, Nigeria for providing lab facilities to conduct this research work.

References

[1] World Health Organisation. National policy on traditional medicine and regulation of herbal medicines: Report of WHO global survey. Geneva: WHO Press; 2005. 5-16p.

[2] Tilburt CJ, Kaptchuk JT. Herbal medicine research and global health: an ethical analysis. *Bulletin of the World Health Organisation* 2008; 86: 577-656

[3] Bandarayanake WM. Quality control, screening, toxicity and regulation of herbal drugs. In: Ahmad I, Aqil F, Owais M (Eds.). Modern phytomedicine: turning medicinal plants into drugs. Weinheim: WILEY-VCH Verlag GmbH & Co, KGaA; 2006. 25-57 p.

[4] Hoffmann D. Healthy digestion: a natural approach to relieving indigestion, gas, heartburn, constipation, colitis and more. (1st Ed) Hoffmann, D. (Ed.). Massachussetts: Storey Publishing LLC; 2000. 1-124 p. ISBN 1580172504

[5] Ukoha OP, Nnamdi OL, Cemaluk CE, Madus PE. Tannins and other phytochemical of the *Samanaea saman* pods and their antimicrobial activities. *African Journal of Pure and Applied Chemistry* 2011; 5: 237-244

[6] Rani V, Deep G, Singh RK, Palle K, Yadav UC. Oxidative stress and metabolic disorders: pathogenesis and therapeutic strategies. *Life Sciences* 2016; 148: 183-193

[7] Harborne JB. Phytochemical methods. In: Harbone, J.B. (Ed.). A guide to modern techniques of plant analysis. London, England: Chapman and Hall Ltd; 1973. 49-188 p. ISBN 9789782462190

[8] Sofowora A. Medicinal plants and traditional medicine in Africa. Ibadan, Nigeria: Spectrum Books Ltd.; 1993. 191-289 p. ISBN 9782462195.

[9] Sharma A, Goyal R, Sharma L. Potential biological efficacy of *Pinus* plant species against oxidative, inflammatory and microbial disorders. *BMC Complementary and Alternative Medicine* 2015; 16(1): 35

[10] Hossain MA, Wakabayashi H, Goda F, Kobayashi S. Effect of immunosuppressant FK506 and d-allose on allogenic orthotopic liver transplantation in rats. *Transplant Proceedings* 2000; 32: 2021-2023

[11] Sui L, Dong Y, Watanabe Y, Hatano N, Tsukamoto I, Izumori K, Tokuda M. The inhibitory effect and possible mechanisms of d-allose on cancer cell proliferation. *International Journal of Oncology* 2005; 27: 907-912

[12] Murata A, Sekiya K, Watanabe Y, Yamaguchi F. A novel inhibitory effect of d-allose on production of reactive oxygen species from neutrophils. *Journal of Bioscience and Bioengineering* 2003; 96: 89-91

[13] Zhao L, Chen J, Su J, Li L, Hu S, Li B, Zhang X, Xu Z, Chen T. In vitro antioxidant and antiproliferative activities of 5-hydroxymethylfurfural. *Journal of Agricultural and Food Chemistry* 2013; 61(44): 10604-10611

[14] Sirigiri CK, Kandru A. GC-MS analysis of biologically active compounds in Canthium parviflorum lam. leaf and callus extracts. *International Journal of ChemTech Research* 2017; 10(6): 1039-1058

[15] Saikarthik J, Saraswathi I, Vijayakumar J, Vijayaraghavan R. Phytochemical analysis of methanolic extract of seeds of Mucuna pruriens by gas chromatography mass spectrometry. *International Journal of Pharmaceutical Sciences and Research* 2017; 8(7): 2916-2921

[16] Parthipan MV, Mohan DN, Sivakama S, Karuppusamy S, Mohan VR. GC-MS analysis of leaf and stem bark of Cleidion nitidum. *Asian Journal of Pharmaceutical and Clinical Research* 2014; 7(2): 1-7

[17] Rahman MM, Ahmad SH, Mohamed MTM, Ab Rahman AZ. (2014). Antimicrobial compounds from leaf extracts of Jatropha curcas, Psidium guajava and Andrographis paniculate. *The Scientific World Journal* 2014; 2014: 1-8

[18] Kumar PP, Kumaravel S, Lalitha C. Screening of antioxidant activity, total phenolics and GC-MS study of Vitex negundo. *African Journal of Biochemistry Research* 2010; 4(7): 191-195.

[19] Takahashi K, Satoh H, Kakuchi T, Miura M, Sasaki A, Sasaki M, Kaga H. Formation kinetics of levoglucosan from glucose in high temperature water. *Chemical Engineering Journal* 2009; 153: 1-3

[20] Wei Y, Chunhua Q. Synthesis of epigoitrin from R-(+)-4-hydroxy-γ-butyrolactone. *Journal of Heterocyclic Chemistry* 2013; 50(6): 1290-1293

[21] Onyeaghala AA, Omotosho IO, Shivashankara, AR. Chemical isolation and characterization of a popular detoxifying herbal remedy Yoyo bitters using GC-MS, NMR and FTIR analysis. *International Research Journal of Pure and Applied Chemistry* 2015; 6(4): 190-200

[22] Kokate CK. Practical pharmacognosy. (3rd ed.). Kokate, C.K. (Ed.). New Delhi, India: Vallabh Prakashan; 1994. 112-120 p.

[23] Anionye JC, Onyeneke EC. Composition and in vitro antioxidant capacity of yoyo bitters. *European Journal of Biological Sciences* 2016; 8(3): 108-115

[24] Sodipo OA, Akiniyi JA, Ogunbamosu JU. Studies on certain characteristics of extracts of bark of Pansinystalia macroceras pierre exbeille. *Global Journal of Pure and Applied Sciences* 2000; 6: 83-87

[25] Atmani D, Nassima C, Dina A, Meriem B, Nadjet D, Hania B. Flavonoids in human health: from structure to biological activity. *Current Nutrition and Food Science* 2009; 5: 225-237

[26] Haslam E. Natural polyphenols (vegetable tannins) as drugs: possible modes of action. *Journal of Natural Products* 1996; 59: 205-215

[27] Traore F, Faure R, Ollivier E, Gasquet M, Azas N, Debrauwer L, Keita A, Timon-David P, Balansard G. Structure and antiprotozoal activity of triterpenoid saponins from Glinus oppositifolius. *Planta Medica* 2000; 66: 368-371

[28] Saxena M, Saxena J, Nema R, Singh D, Gupta A. Phytochemistry of medicinal plants. *Journal of Pharmacognosy and Phytochemistry* 2013; 1(6): 168-182

[29] Prassas I, Diamandis EP. Novel therapeutic applications of cardiac glycosides. *Nature Reviews Drug Discovery* 2008; 7: 926-935

[30] Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology* 2005; 4(7): 685-688

[31] Kim SY, Kim JH, Kim SK, Oh MJ, Jung MJ. Antioxidant activities of selected oriental herb. *Journal of the American Oil Chemists' Society* 1994; 71: 633-640

[32] Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry* 2005; 53(10): 4290-4302

[33] Duan XJ, Zhang WW, Li WXM, Wang BG. Evaluation of antioxidant property of extract and fraction obtained from red alga Polysiphonie urcelata. *Food Chemistry* 2006; 95: 1022-1037