Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2021; 10(3): 154-164
Received: 30-03-2021
Accepted: 29-04-2021

Ogbonnia SO
Department of Pharmacognosy, Faculty of Pharmacy, Chukwuemeka Odumegwu Ojukwu University, Igbariam, Nigeria

Ginikachukwu O
Department of pharmacognosy, Faculty of pharmaceutical Sciences, Enugu State University of Science and Technology, Agbani, Nigeria

Ezemenahi SI
Department of Family Medicine, Nnanmdi Azikwe University Awka, Nigeria.

Okeke AI
Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, Chukwuemeka Odumegwu Ojukwu University, Igbariam, Nigeria.

Ota Duncan
Department of Physiology, College of Medicine, University of Lagos, Lagos, Nigeria

Corresponding Author:
Ogbonnia SO
Department of Pharmacognosy, Faculty of Pharmacy, Chukwuemeka Odumegwu Ojukwu University, Igbariam, Nigeria

Preliminary Phytochemistry, Antioxidant activities and GC/MS of the most abundant compounds of different solvents fractions of the Plant *Bridelia ferruginea* Benth used locally in the management of Diabetes

Ogbonnia SO, Ginikachukwu O, Ezemenahi SI, Okeke AI and Ota Duncan

Abstract
Background: Plants and their derived products have been employed since prehistoric era for their therapeutics and health benefits, in addition to their invaluable food and nutritive values. *Bridelia ferrugihea* is one of such plants endowed with poly-therapeutics activities and has been employed extensively by traditional herbalists in Nigeria for management of various sickness.
Objectives: This study carried out the preliminary phytochemical screening, evaluated antioxidant activities, chromatographic and GC/MS of different solvents extracts of *Bridelia ferruginea* leaf.
Materials and Methods: Antioxidant activities, phytochemical screening, chromatographic analysis and GC/MS analysis were performed using standard methods.
Results: The phytochemical screening revealed the presence of various secondary metabolites such as alkaloids, flavonoids amongst others. The extract demonstrated some antioxidant activities. The GC/MS of various fractions revealed Palmitic acid, vinyl ether, E-heptadecenal,4.4,6a,6b,8a,11,12,14b-octamethy l,12a14,14a,14b-octadecahydro-2h-picen-3–one,:2,2,6-trimethyl-1-(2-methylcyclobut-2-enyl)-hepta-4,6 dien-3-one;Hexadecanoicacid ethyl ether; Phytol; Neophytadiene and 2-Pentadecanone,6,10,14 trim ethyl as the most abundant constituents.
Conclusion: The study revealed that the multiple physiological and pharmacological and antioxidant activities of the plant extract could be attributed to the presence of secondary metabolites justifying local use of the plant and the claims by the traditional healers that the plant drug could be used to manage some disease states that might not be easily managed by orthodox medicines.

Keywords: Phytochemistry screening, antioxidant, chromatography and GC/MS

Introduction
Plants and their derived products have served from the outset as veritable sources of food for the sustenance of lives of humans and animals. The discovery since prehistoric era that plants' products, in addition to their nutritive values, could also serve as useful therapeutic weapons against various human, animal and even plant diseases has made plants a sine qua non to human and animal lives (Ogbonnia *et al.*, 2008; Abo *et al.*, 2008) [54, 1]. Medicines obtained from plants are popularly known as "Herbal drug" or "phytomedicine" and is currently renowned and recognized as the most common form of alternative medicine. Herbal medicine is used by about 60% of the world population both in the developing and in the developed countries where modern medicines are predominantly used (Ogbonnia *et al.*, 2009; Adesoka *et al.*, 2010) [55, 3]. The use of these plant remedies especially in the form of teas or extracts for the treatment of various diseases is gaining increasing popularity, making them the main stay of health care system, especially among the rural populace in the developing countries (Ali *et al.*, 2006) [7]. Their increasing popularity could be attributed to their advantages of being efficacious, cheap and also could be employed to manage diseases that orthodox medicines cold only proffer palliative solutions.
The physiological and pharmacological activities of the plant drugs could be attributed to secondary metabolites or "natural products" present in them which could be alkaloids, products of acetate, mevalonic acid pathways such as terpenes and terpenoids, flavonoids or products of shikimic acid pathways (Ebong *et al.*, 2008; Cimanga *et al.*, 1999) [11, 10].
Several orthodox drugs currently used for the management of some disease states have been found to proffer only palliative measures and might be associated with serious side-effects but in diabetes, they are often unable to lower glucose concentrations to within the normal range, or to reinstate a normal pattern of glucose homeostasis. The orthodox drugs even when they are effective, their uses are often restricted by their pharmacokinetic properties, secondary failure rates and accompanied undesirable effects.

For these reasons, there is ardent need to intensify search for an acceptable, cheap and safe drug sources and, therefore, alternative therapy of plants and plant derived products are being considered as the best options (Ogbonnia *et al.*, 2010) [57]. Plants also have been found to contain secondary metabolites or natural products with antioxidant activities.

Oxidative stress emerges from the existence of free radicals (molecules possessing an unpaired electron) and reactive oxygen species (ROS), formed in normal physiological processes but become deleterious when they are not quenched by a cascade of antioxidant systems. Oxidative stress has been considered as a major contributing factor associated with the β-cell damage (Oyaizu, 1986: Hutching *et al.*, 1996; Quilliot *et al.*, 2005; Adika *et al.*, 2011) [65, 26, 91, 4] and can result either from an overproduction of ROS or from the inactivation of the antioxidants, thereby shifting the balance in favour of oxidative stress. *Bridelia ferruginea* Benth. (Euphorbiaceous) is one of such plant based drugs being investigated for the management of oxidative stress especially in diabetes.

Bridelia ferruginea Benth. (Euphorbiaceous) is commonly found in Savannah regions (Ekanem *et al.*, 2008) [15]. Its bark extract has been used for the coagulation of milk and also lime juice for the formulation of a traditional gargle "Egun Efu". It is also reported of having potential for water treatment and chemo preventive potentials. Other reported activities of the bark extract include trypanocidal, molluscidal (Adeoye *et al.*, 1988) [2] antimicrobial (Ndukwe *et al.*, 2005) [51] and anti-inflammatory (Olajide *et al.*, 1999) [63]. Hence, in this study, we carried out systematic investigation of the preliminary phytochemical screening, antioxidant activities, thin layer chromatographic identification and separation of the fractions and GC/MS characterization of the fractions of *Bridelia ferruginea*, along with identification of compounds in the most solvent fractions.

Materials and methods

Preparation of plant extracts

Fresh leaves of *Bridelia ferruginea* Benth (Euphobiaceae) were bought from a local market in Abeokuta and were identified and authenticated by O.O. Oyebanji, a taxonomist at Department of Botany, University of Lagos. A voucher specimen with voucher number LUH 7550 was deposited at the herbarium of the Department of Botany for reference purposes. They were dried under room temperature and ground to coarse powder. The powdered leaves were extracted using cold macerations in 90% ethanol for two weeks. A rotary evaporator was used to concentrate the extract. The crude extract obtained was further lyophilized at -4 ^{0}C to obtain 88.20g dry mass (7.35% yield).

Reagents and chemicals

General chemicals used include organic solvents (n-Hexane, Ethyl acetate and Methanol). Other chemicals used were 3, 5-Dinitrosalicylate reagent (DNS) and sodium carbonate. All were obtained from Merck and were of analytical grade.

Preliminary Phytochemical Screening

Phytochemical screening of the extract for the presence of secondary metabolites was performed with standard methods described by (Farnsworth 1966; Sofowora 1993; and Harborne 1998; Houghton and Raman 1998; Saidu *et al.*, 2012) [17, 76, 21, 24, 71].

Test for Anthraquinones

Bontr**ä**ger's Test: 5 mL of chloroform was added to 0.5 g of the plant extract. The resulting mixture was shaken for 5 minutes after which it was filtered. The filtrate was then shaken with equal volume of 10% ammonia solution. The presence of a bright pink colour in the aqueous layer indicated the presence of Anthraquinones.

Modified Bonträger's Test: 0.5g of crude drug was boiled with 2ml of dilute sulphuric acid and 2ml of ferric chloride for 5 minutes and filtered. Equal volume of dichloromethane was added to partition the filtrate. 5ml of dilute ammonia was added to the organic phase and shaken for few seconds. The formation of rose pink to red colour indicated the presence of Anthraquinones.

Test for Reducing Sugar (Fehlings Solution Test)

To about 1 g of each plant extract in the test tube, 10 mL distilled water was added and the mixture boiled for 5 min. The mixture was filtered while hot and the cooled filtrate made alkaline to litmus paper with 20% sodium hydroxide solution. To 2 ml of the water extract was added 5 ml of a mixture of equal volume of Fehling's solution A and B and boiled on a water bath for 2 minutes. A brick-red colour solution indicates the presence of reducing sugar.

Test for saponin

2 g of plant extract was boiled in 20 mL of distilled water in a water bath and filtered. Next, 10 mL of the filtrate was mixed with 5 mL of distilled water and shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously again and then observed for the formation of emulsion as an indication of saponin

Test for tannins

Ferric chloride test: 0.5 g of plant extract was boiled in 20 mL of water in a test tube and filtered. Few drops of 0.1% ferric chloride was added and observed for a brownish green or blue black colouration as an indication of tannins.

Bromine water test: 10 ml of bromine water was added to the 0.5 g aqueous extract. Decolouration of bromine water showed the presence of tannins.

Test for Terpenoids

0.5g of plant extract was mixed with 2 ml chloroform and 3 ml H_2SO_4 was carefully added to form a layer. A reddish brown colouration of the interface was an indication of terpenoids.

Tests for flavonoids

Shinoida Test: Pieces of magnesium ribbon and HCL concentrated were mixed with aqueous crude plant extract after few minutes and pink colour showed the presence of flavonoid.

Alkaline reagent test: 2 ml of 2.0% NaOH mixture was mixed with aqueous plant crude extract; concentrated yellow colour was produced, which became colourless when we added 2 drops of diluted acid to mixture. This result showed the presence of flavonoids.

Tests for steroids

Liebermann-Buchard's Test: Extract was treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added from the sides of the test tube. Formation of

brown ring at the junction of the two layers and upper layer turning green indicates the presence of steroids.

Test for Cardiac glycosides

Keller-Killani Test (For Deoxy Sugars)

5 ml of extract was mixed with 2 ml of glacial acetic acid containing one drop of 2% ferric chloride ($FeCl_3$) solution, followed by the addition of 1 ml concentrated sulphuric acid. Formation of brown ring at the interface indicates the presence of deoxy sugar. A violet ring may appear beneath the brown ring, while in the acetic acid layer, a greenish ring may also form just gradually throughout the layer.

Kedde's Test: (For Lactone Rings)

Crude drug was extracted with chloroform and evaporated to dryness. One drop of 90% alcohol and 2 drops of 3,5-dinitobenzoic acid in 90% alcohol was added. The solution was made alkaline with 20% NaOH solution. Formation of purple colour indicates the presence of β-unsaturated lactone rings

Test for Coumarins: 0.5 g of the moistened various extracts was taken in a test tube. The mouth of the tube was covered with filter paper treated with 1 N NaOH solution. Test tube was placed for few minutes in boiling water and then the filter paper was removed and examined under the UV light for yellow fluorescence indicated the presence of Coumarins.

Tests for Alkaloids

A 20 ml of 5% sulphuric acid in 50% ethanol was added to about 2 g of the sample. This was heated on a boiling water bath for 10 minutes, cooled and filtered. The filtrate was transferred into four test tubes each containing 2 ml of the filtrate and used for the following tests.

a) To the first test tube, few drops of Dragendorff's reagent (a solution of bismuth iodide in Potassium iodide) was added and homogenized. Formation of brick red precipitate indicated the presence of alkaloids.
b) About two drops of Wagner's reagent (a solution of iodine in Potassium iodide) was added to the second test tube and swirled for few seconds. Brownish- red precipitate indicated the presence of alkaloids.
c) About two drops of Mayer's reagent (a solution of Mercury iodide in Potassium iodide) was added to the third test tube and homogenized for few seconds. A creamy (dirty white) precipitate indicated the presence of alkaloids.

Test for Carbohydrate (Molisch Test)

A small quantity (0.1 g) of the extract was boiled with 2 ml of distilled water and filtered. To the filtrate, few drops of naphtol solution in ethanol (Molisch's reagent) were added. Concentrated

Sulphuric acid was then gently poured down the side of the test tube to form a lower layer. A purple interfacial ring indicates the presence of carbohydrate.

Test for cyan genetic Glycosides

2g of the crude drug was placed in a conical flask and moistened with few drops of water. A piece of picric acid paper was moistened with sodium carbonate solution (5% aqueous) and suspended by means of cork in the neck of the flask and warmed gently at about 37 °C. The change in colour is observed. Hydrogen cyanide is liberated from cyan genetic glycoside by the enzyme activity and reacts with sodium picrate to form the reddish purple sodium isopicrate.

Antioxidant activities

Nitric oxide scavenging activity assay: The compound sodium nitroprusside is known to decompose in aqueous solution at physiological pH (7.2) producing NO•. Under aerobic condition, NO• reacts with oxygen to produce stable products (nitrate and nitrite), which can be determined using Griess reagent. The absorbance of the chromophore that formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with Naphthylethylenediamine dihydrochloride can be immediately read at 550 nm. A 4 ml sample of plant extract or standard solution of different concentrations (20, 40, 60, 80, 100µg/ml) were taken in different test tubes and 1 ml of Sodium nitroprusside (5mm in phosphate buffered saline) solution was added into the test tubes. They were incubated for 2 hours at 30 °C to complete the reaction. A 2 ml sample was withdrawn from the mixture and mixed with 1.2 ml of Griess reagent (1% Sulphanilamide, 0.1% naphthylethylene diamine dihydrochloride in 2% H_3PO_4). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with napthylethylene diamine was measured at 550 nm. Ascorbic acid was used as standard. The percentage (%) inhibition activity was calculated from the following equation

$$[(A_0 - A_1)/A_0] \times 100.$$

Where, A_0 is the absorbance of the Control and A_1 is the absorbance of the extract or standard

DPPH radical scavenging activity assay: The free radical scavenging activity of the extract, based on the scavenging of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was estimated according to the procedure described by. An aliquot of 0.5 ml of extract in ethanol (95%) at different concentrations (20, 40, 60, 80, 100µg/ ml) was mixed with 2.0 ml of reagent solution (0.004 g of DPPH in 100 ml methanol).The control contained only DPPH solution in place of the sample while methanol was used as the blank. The mixture was vigorously shaken and left to stand at room temperature. After 30 minutes the decrease in absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517nm. The scavenging effect was calculated using the expression: % inhibition = $\frac{[A_0 - A_1]}{A_0} \times 100$

Where A_0 is the absorption of the blank sample and A_1 is the absorption of the extract.

Reducing power assay

Reducing power was determined by the method prescribed by Oyaizu *et al.* 8. The sample in 1ml of methanol at various concentrations was mixed with a phosphate buffer (5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (5ml, 1%), and the mixture was incubated at 50 °C for 20 min. Next, 5ml of trichloroacetic acid (10%) were added to the reaction mixture, which was then centrifuged at 3000 RPM for 10 min. The upper layer of the solution (5ml) was mixed with distilled water (5ml) and ferric chloride (1ml, 1%), and the absorbance was measured at 700 nm. A stronger absorbance indicates increased reducing power.

Fractionation of the crude extract

Vacuum liquid chromatography

The VLC column was dry packed with silica gel (Kiesegel 60G, Merck, Germany). Care was taken so that the packing was kept uniform and making an evenly flat surface. The crude extract to be worked on was first mixed with some silica gel and applied as dry powder onto the top of the well packed VLC. Elution was done using various proportions of a solvent gradient of increasing polarity, starting with 100 % *n*-hexane (1L) then EtOAc (1L) and finally MeOH (1L). The fractions collected were concentrated and the percentage yield of each fraction determined

Gas Chromatography-Mass Spectrometric (GC-MS) Analysis

The crude, hexane, ethyl acetate and methanol fractions were further subjected to GC-MS analysis. The analysis was conducted with an Agilent Technologies 6890 GC coupled with an Agilent 5973 mass selective detector and driven by Agilent Chemstation software (Agilent Technologies, USA). A DB-5SIL MS capillary column was used (30 m x 0.25 mm I.D., x 0.25µm film thickness). The carrier gas was ultra-pure helium at a flow rate of 0.7 mL min-1 and a linear velocity of 37 cm s-1. The injector temperature was set at 250 °C. The initial oven temperature was 60 °C, which was programmed to 280 °C at the rate of 10 °C min-1 with a hold time of 3 min. Injections of 2µL were made in the split less mode with a manual split ratio of 20:1. The mass spectrometer was operated in the electron ionization mode at 70 eV and electron multiplier voltage at 1859 V. Other MS operating parameters were as follows: ion source temperature 230 °C, quadrupole temperature 150 °C, solvent delay 4 min and scan range 50-700amu. Compounds were identified by direct comparison of the retention times and mass fragmentation pattern with those from the National Institute of Standards and Technology (NIST) library.

Statistical analysis

Data analysis was done using Graph Pad Prism 7. One way analysis of variance (ANOVA) was used to compare means. One–way ANOVA was done followed by Dennett's multiple comparisons test of treated groups with control. The results were expressed as Mean ± SEM. Level of significance was set at 95% or $p < 0.05$ and 99% or $p < 0.01$

Results

Preliminary phytochemical screening

Results of the preliminary phytochemical screening of the crude extract is as shown in the table below.

Table 1: Phytochemical Constituents of *Bridelia ferruginea* Benth

Phytochemical constituent	Amount present
Alkaloids	+
Flavonoids	++
Phenols	+
Tannins	++
Saponin Glycosides	++
Cyanogenetic glycosides	-
Cardiac Glycosides	++
Reducing sugars	+
Steroids	_
Terpenoids	++
Anthraquinones	-

++ Abundantly present, + slightly present, - absent

Ant-oxidant activity

The anti-oxidant activities of *Bridelia ferruginea* leaf extract and fractions are as shown the tables 4.5, 4.6 and 4.7 below. All fractions of the leaf extract displayed good anti-oxidant activity with the least being the crude extract.

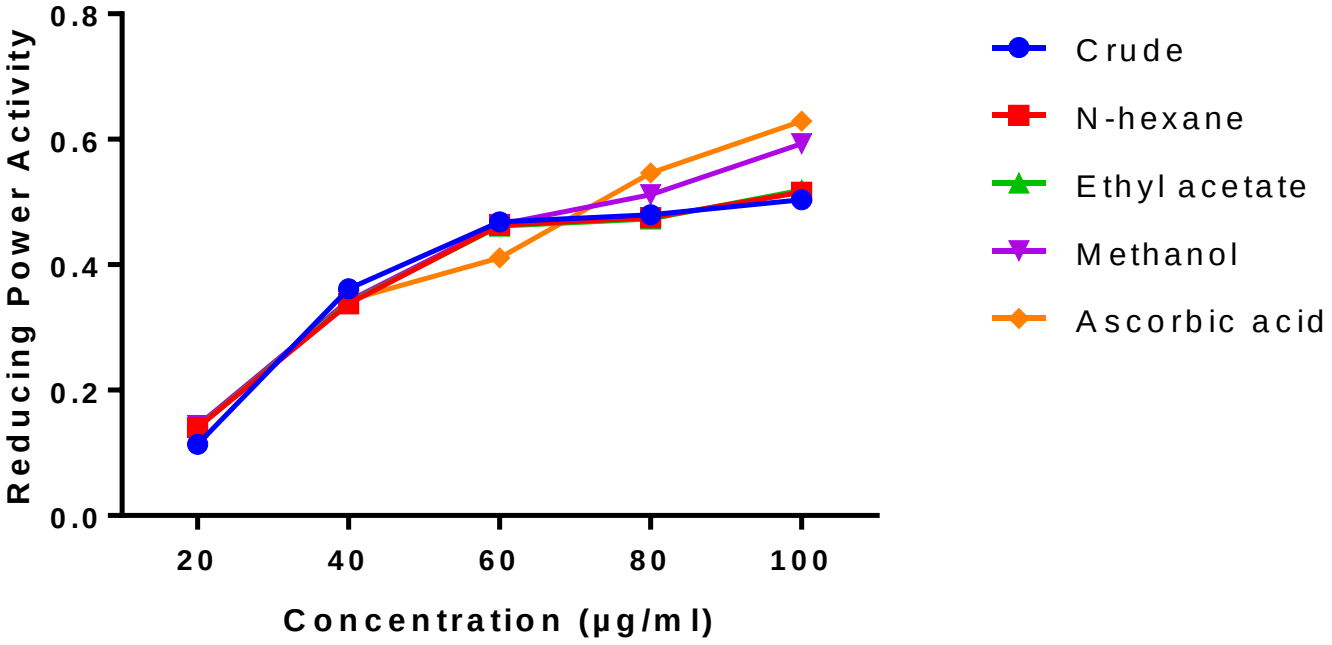


Fig I: Reducing Power Anti-oxidant Activity of *Bridelia ferruginea*

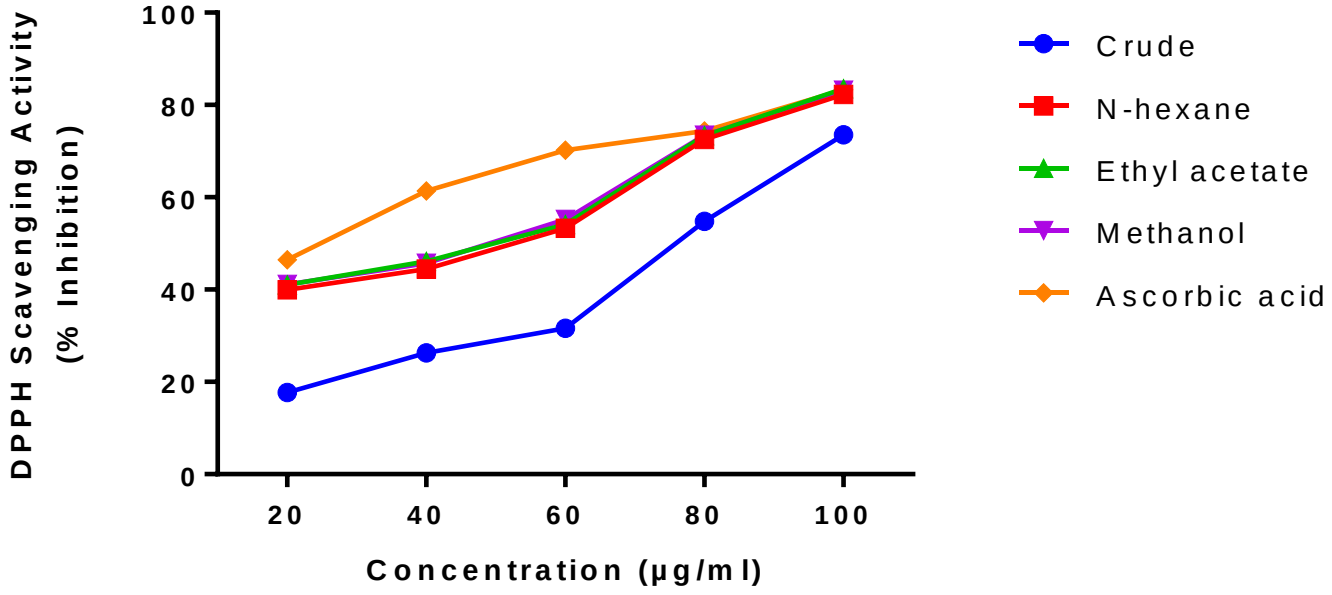


Fig II: DPPH Radical Scavenging Activity of *Bridelia ferruginea*

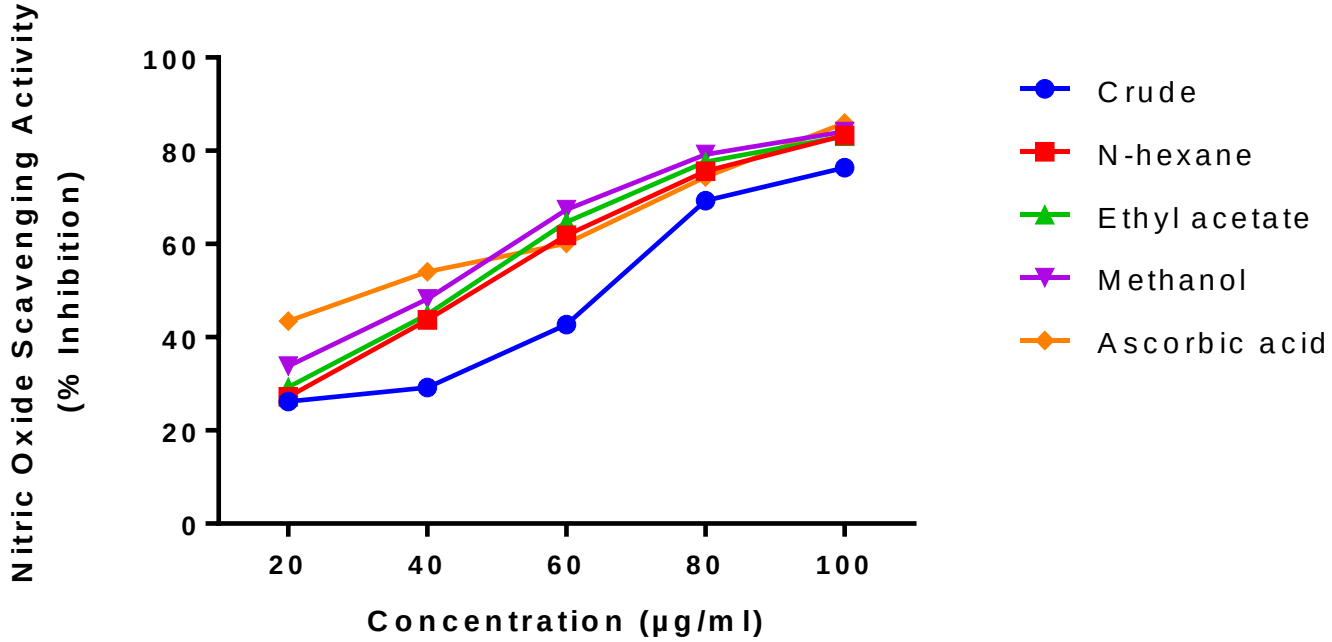


Fig III: Nitric oxide scavenging activity of *Bridelia ferruginea*

GC/MS analysis of leaf extracts of *Bridelia ferruginea*

The GC/MS analysis of the leaf extracts Bridelia ferruginea revealed the presence of flavonoids, sesqiuterpenes, tetraterpenes, modified triterpenoid compounds etc. Methanol fraction of *Bridelia ferruginea* revealed hexadecanoic acid ethyl ester (38.17%) as the most abundant fatty acid present. Other major fatty acids are Pentadecanoic acid (15.21%), E-15-Heptadecenal (25.72%) and E-9-Ocatadecanoic acid Ethyl Ester (3.97%). Other compounds identified are Divinylmethyl (acetoxymethyl) Silane (10.0%) and Indolizine, 2-(4-methylphenyl) (6.93%). The chemical composition of the ethyl acetate fraction obtained from *Bridelia ferruginea* using GC-MS revealed that the major compounds are fatty acid esters (Table 4.15). Hexadecanoic acid methyl ester also known as methyl palmitate (22.96%) is the major fatty acid ester obtained in the ethyl acetate fraction. Other abundant fatty acid esters include ethyl Oleate (3.35%) Octadecanoic acid, methyl ester (5.35%) Linoleic Acid Ethyl Ester (3.57%). Other constituents are 1, 3-dimethylbenzene (3.40%), P-xylene (4.15%), Phytol (19.38%), Bis-(2-ethylhexyl) phthalate (3.49%), Alpha-Tocopherol (16.60%) and Squalene (22.11%). The hexane fraction of *Bridelia ferruginea* revealed the following compound; Chloromethoxy, ethane (1.27%), Neophytadiene (5.43%), 2-Pentadecanone, 6,10,14-trimethyl (5.93%), 1-Methoxy-3-(2-hydroxyethyl) Nonane (2.40%), 1,19-Eicosadiene (3.01%), Tetradecanoic acid, ethyl ester (5.78%), 3-Cyclopentylpropionic acid, ethyl Esther (0.21%).

Table 2: Phytoconstituents Identified in the Hydro-Ethanolic Fraction of Bridelia ferruginea using GC/MS

Peak no	Retention Time	% Relative Abundance	Component	Molecular weight
1.	28.693	13.78	4,4,6a,6b,8a,11,12,12a,14b-Octamethyl,12a,14,14a,14b-octadecahydro-2H-picen-3-one	
2.	29.494	15.07	2,2,6-Trimethyl-1-(2-methyl-cyclob ut-2-enyl)-hepta-4,6-dien-3-one	218
3.	30.375	8.81	Ergost-25-ene-3,5,6,12-tetrol, beta.,5.alpha.,6.beta.,12.beta.)-	450
4.	30.770	0.38	m-Camphorene	272
5.	31.148	1.47	9,19-Cyclolanost-24-en-3-ol,	440
6.	31.617	9.43	Friedelan-3-one	426
7.	32.944	2.57	1,4-Bis(Trimethylsilyl)benzene	194
8.	33.791	3.13	Silicic acid, diethyl Bis(trimethylsilyl) ester	290
9.	34.358	2.72	Benzo[h]Quinoline, 2,4-dimethyl-	207

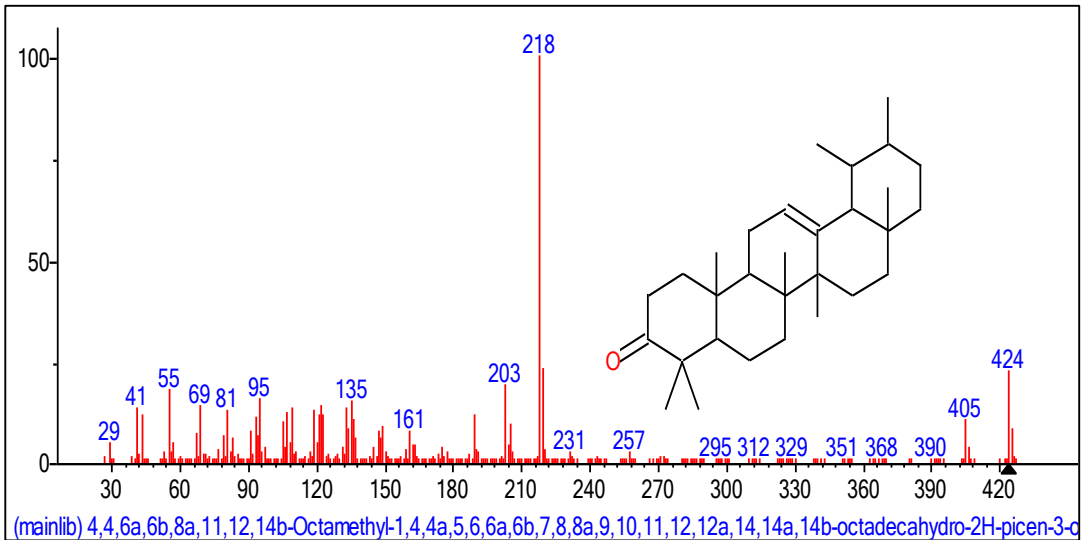


Fig V(AI): The most abundant chemical component of this fraction

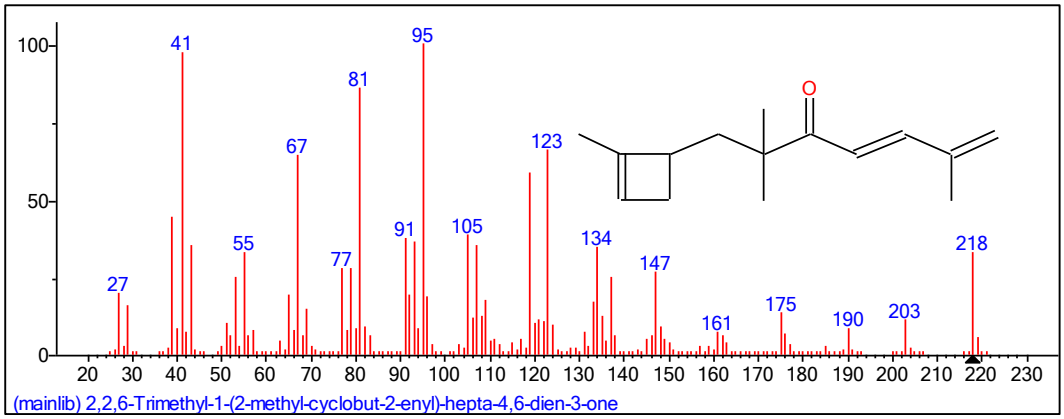
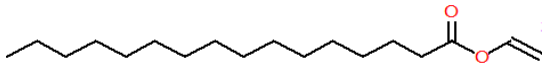


Fig V(AII): The second most abundant chemical component of this fraction

Table 3: Phytoconstituents Identified in Methanol Fraction of *Bridelia ferruginea* using GC/MS

Peak no	Retention Time	% Relative Abundance	Component	Molecular weight
1.	17.27	10.0	Divinylmethyl(acetoxymethyl)Silane	170
2.	19.64	15.21	Pentadecanoic acid	270
3.	20.71	38.17	Palmitic Acid Vinyl Ester	284
4.	22.77	25.72	E-15-Heptadecenal	252
5.	23.62	3.97	E-9-Ocatadecanoic acid Ethyl Ester	310
6.	31.14	6.93	Indolizine, 2-(4-methylphenyl)	207



Palmitic acid vinyl ester

Fig V(BI): The abundant chemical component of the fraction

E-15-heptadecenal

Fig V(BII): The abundant chemical component of the fraction

Table 4: Phytoconstituents Identified in Ethyl Acetate Fraction of *Bridelia ferruginea* using GC/MS

Peak no	Retention Time	% Relative Abundance	Component	Molecular weight
1.	4.66	3.40	1,3-dimethylbenzene	106
2.	4.71	4.15	P-xylene	106
3.	20.75	16.39	Hexadecanoic Acid Ethyl Ester	284
4.	22.828	19.38	Phytol	296
5.	23.57	3.57	Linoleic Acid Ethyl Ester	308
6.	23.66	3.35	Ethyl Oleate	310
7.	24.05	5.35	Octadecanoic Acid Ethyl Ester	312
8.	28.69	3.49	Bis-(2-ethylhexyl) phthalate	390
9.	28.96	2.21	(2,3-Diphenylcyclopropyl)methyl phenyl sulphoxide	332
10.	31.17	22.11	Squalene	411
11.	33.67	16.60	Alpha-Tocopherol	430

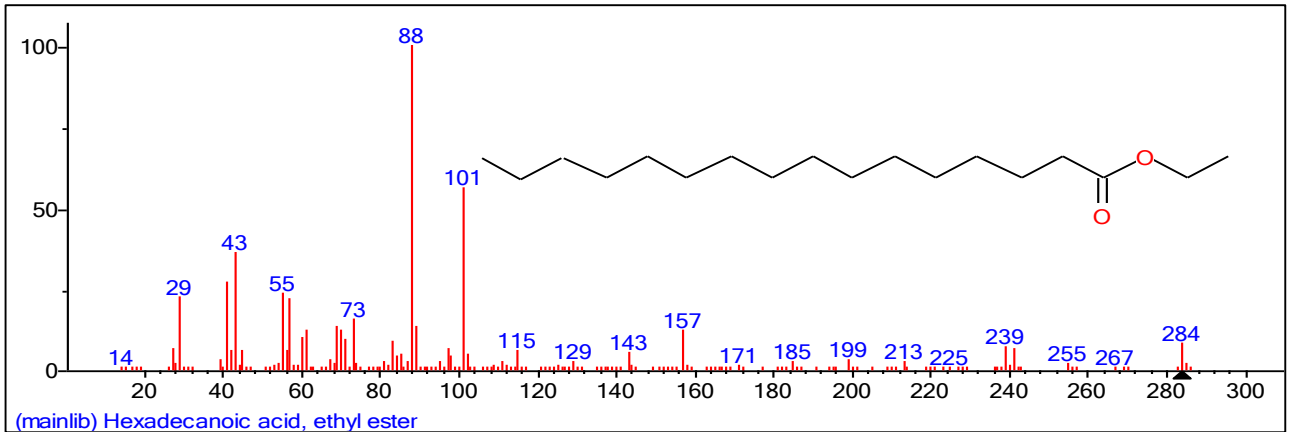


Fig V(CI): The most abundant chemical component of the fraction

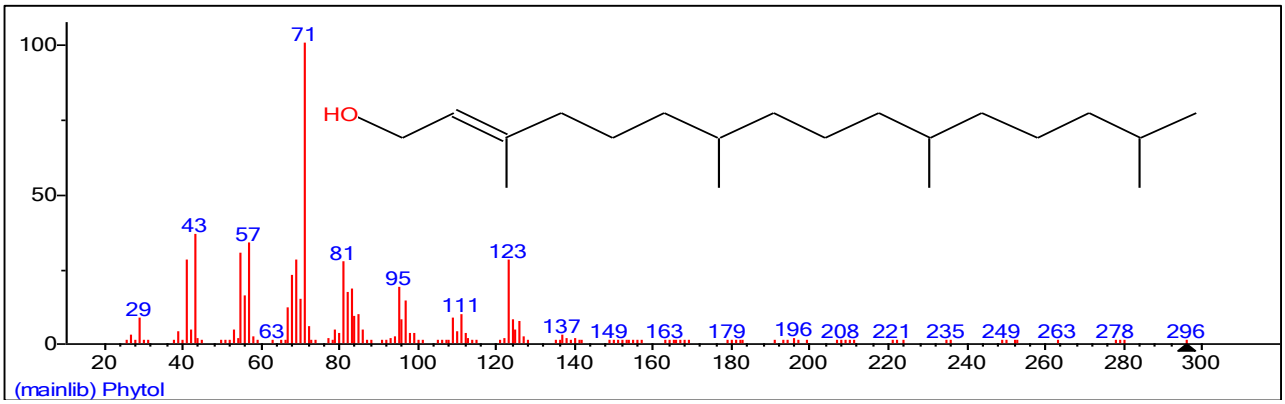


Fig V(CII): The second most abundant chemical component of the fraction

Table 5: Phytoconstituents Identified in n-hexane Fraction of *Bridelia ferruginea* using GC/MS

Peak no	Retention Time	% Relative Abundance	Component	Molecular weight
1.	4.79	1.27	Chloromethoxy,ethane	93, 96
2.	15.50	5.43	Neophytadiene	278
3.	15.85	5.93	2-Pentadecanone, 6,10,14-trimethyl	268
4.	16.13	2.40	1-Methoxy-3-(2-hydroxyethyl)nonane	202
5.	16.59	3.01	1,19-Eicosadiene	278
6.	17.59	5.78	Tetradecanoic acid, ethyl ester	256
7.	18.23	10.53	Neophytadiene	278
8.	18.41	8.19	2-Pentadecanone, 6,10,14-trimethyl	268
9.	18.61	2.12	1,13-Tetradecadiene	194
10.	18.69	0.21	3-Cyclopentylpropionic acid, ethyl esther	170
11.	18.91	1.43	Hexadecanoic acid, ethyl ester	284

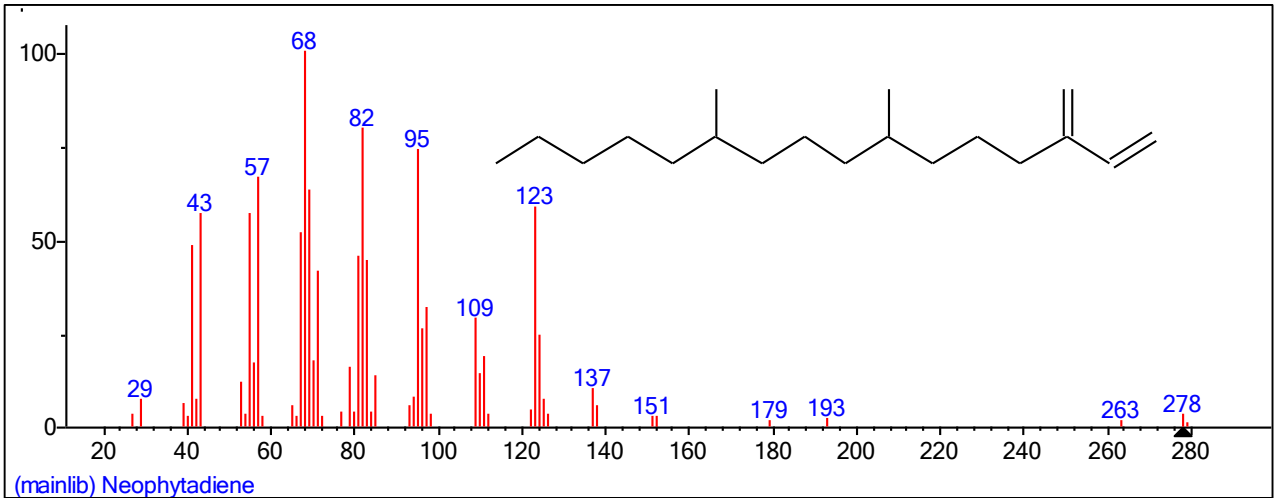


Fig V(DI): The most abundant chemical component of the fraction

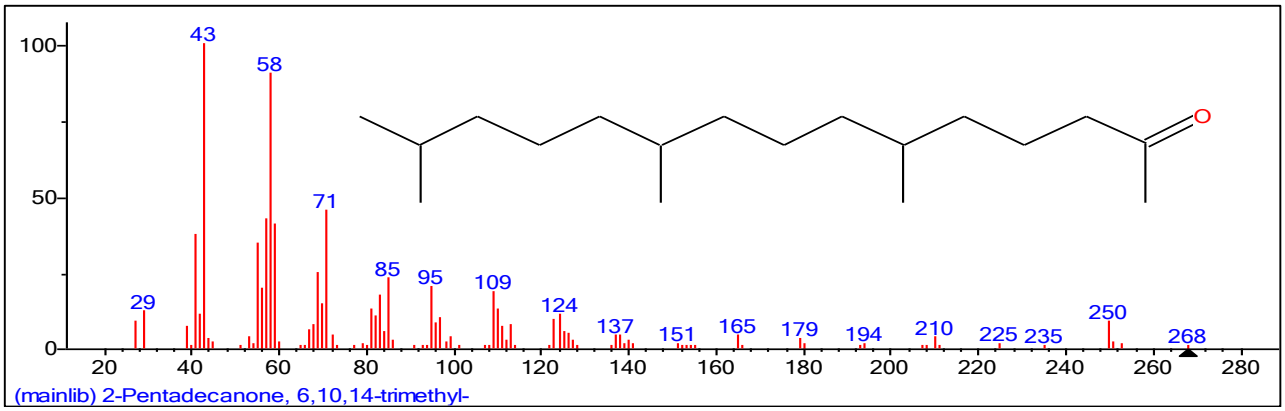


Fig V(DII): The second most abundant chemical component of the fraction

Results and discussion

Plant or herbal drugs have received greater attention as alternative to clinical therapy in recent times leading to subsequent increase in their demand (Sushruta *et al.*, 2006) [77]. In rural communities, the exclusive use of herbal drugs, prepared and dispensed by herbalists without formal training, for the treatment of diseases is still very common. Experimental screening method is, therefore, required for the evaluation of the claimed therapeutic activity, and to ascertain the safety and efficacy of these herbal products as well as to establish their active constituents (Ogbonnia *et al.*, 2008) [54].

Phytochemical screening was observed the presence of primary metabolites especially the reducing sugars which might have eventually metabolized to the end products which in the presence of different enzymes might have given rise to various secondary metabolites such as alkaloids, flavonoids, tannins, Saponins and terpenoids found in the plant. The plant, *Bridelia ferruginea* Benth, is currently being employed by the traditional herbalist healers locally to manage various disease states that are recalcitrant to orthodox drugs and the Polytherapeutic activities of the plant could be attributed to the presence of multiple secondary metabolites such as alkaloids, flavonoids, Saponins and terpenoids present in the plant. The flavonoids have been associated with antioxidant activities and is employed to manage various disease states with different oxidative stress aetiologies.

The various solvent fractions of the *Bridelia ferruginea* leaf extract demonstrated to exhibit better antioxidant activities compared to the crude extract of the plant. In the nitric oxide antioxidant evaluation ascorbic acid and methanol fractions respectively were shown to be more active than n-hexane and ethyl acetate fractions as antioxidants. In the reducing power antioxidant assessment crude extract showed the least activity, n-hexane, methanol and ethyl acetate fractions respectively appeared to same level of activities while ascorbic acid demonstrated to have the greatest activities. The DPPH Free radical scavenging activity crude extract has the least activity while n-hexane, ethyl acetate, methanol and ascorbic acid fractions respectively showed similar activities.

GC/MS results show the following as the abundant chemical components of different fractions respectively. The GC/MS of various fractions revealed Palmitic acid vinyl ether, E-heptadecenal,4.4,6a,6b,8a,11,12,14b-octamethyl,12a14,14a, 14b-octadecahydro-2h-picen-3–one,:2,2,6-trimethyl-1-(2-methylcyclobut-2-enyl)-hepta-4,6dien-3-one; Hexadecanoic acid ethyl ether; Phytol; Neophytadiene and 2-Pentadecanone,6,10,14 trim ethyl as the most abundant constituents.

Conclusion

The study has undoubtedly observed that *Bridelia ferruginea* exhibited potential antidiabetic activity in alloxan-induced diabetic rats, giving credence to the traditional claims. Also in vitro studies demonstrated that *Bridelia ferruginea extracts* inhibited enzymes involved in carbohydrate metabolism and also had promising antioxidant activities which aids its antidiabetic activities.

References

1. Abo KA, Fred-Jaiyesimi AA, Jaiyesimi AEA. Ethno botanical studies of medicinal plants used in the management of diabetes mellitus in South Western

Nigeria, Journal of Ethno pharmacology. 2008;115:67–71.
2. Adeoye AO, Abaeli AM & Owowumi CJ. Antimicrobial activity of Bridelia ferruginea, In: Book of abstract of the symposium on drug production from natural products Ile-Ife, 1988, 24.
3. Adesokan A, Akanji MA, Adewara GS. Evaluation of hypoglycaemic efficacy of aqueous seed extract of Aframomum melegueta in alloxan-induced diabetic rats, Sierra Leone Journal of biomedical research. 2010;2:91–94.
4. Adika OA, Madubunyi II, Asuzu IU. Antidiabetic and antioxidant effects of the methanol extract of Bridelia micrantha (Hochst) Baill (Euphorbiaceous) leaves on alloxan-induced diabetic albino mice comparative Clinical Pathology. 2011;10:1205–120.
5. Ahmed I, Adeghate E, Cummings E, Sharma AK, Singh J. Beneficial effects and mechanism of action of Momordica Charantia juice in the treatment of streptozotocin-induced diabetes mellitus in rats, Molecular and Cellular Biochemistry. 2004;261:63–70.
6. Akinola OB, Caxton-Martins EA, Dini L. Chronic treatment with Ethanolic extract of the leaves of Azadirachta indicia Ameliorates lesions of pancreatic islets in streptozotocin diabetes, International Journal of Morphology. 2010;28:291–302.
7. Ali H, Houghton PJ and A Soumyanath. "α-Amylase inhibitory activity of some Malaysian plants used to treat diabetes with particular reference to Phyllanthus amarus," Journal of Ethno pharmacology. 2006;107(3):449–455.
8. Bekele T. Antidiabetic activity and phytochemical screening of crude extracts of Stevia rebaudiana Bertoni and Ajuga remota Benth grown in Ethiopia on alloxan-induced diabetic mice. (Doctoral dissertation, Department of Pharmaceutical Chemistry, School of Pharmacy, Addis Ababa University, 2008.
9. Bhandari MR, Anurakkun NJ, Hong G, Kawabata J. α-Glucosidase and α-amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (Bergenia cilata Haw.) Food Chem, 2008;106:247.
10. Cimanga K, De Bruyne T & Apers S. Complement-inhibiting constituents of Bridelia ferruginea stem bar. Plantae Med, 1999;65:213-217.
11. Ebong PE, Atangwho IJ, Eyong EU, Egbung GE, The antidiabetic efficacy of combined extracts from two continental plants: Azadirachta indica (A. Juss) (Neem) and Vernonia amygdalina (Del.) (African Bitterleaf), American Journal of Biochemistry and Biotechnology. 2008;4:239–244.
12. Egunyomi A, Gbadamosi IT, Animashahun MO, Hypoglycaemic activity of the ethanol extract of Ageratum Conyzoides Linn. Shoots on alloxan-induced diabetic rats, Journal of Medicinal Plants Research. 2011;5:5347–5350.
13. Eidi A, Eidi M, Esmaeili E. Antidiabetic effect of garlic (Allium Sativum L.) in normal and Streptozotocin-induced diabeticrats Phytomedicine. 2006;13:624–629.
14. Ejike CC, Awazie SO, Nwangozi PA, Godwin CD, Synergistic postprandial blood glucose modulatory properties of Vernonia Amygdalina (Del.), Gongronema latifolium (Benth.) and Occimum gratissimum (Linn.) aqueous decoctions, Journal of Ethno pharmacology. 2013;149:111–116.
15. Ekanem JT, Kolawole OM & Abbah OC. Trypanocidal Potential of Ethanolic extracts of Bridelia ferruginea benth bark in Rattus novergicus, Afr J Biochem Res. 2008;2(2):045-050.
16. Etxeberria U, De la Garza AL, Campión J, Martínez JA, Milagro FI. Antidiabetic effects of natural plant extracts via inhibition of carbohydrate hydrolysis enzymes with emphasis on pancreatic α – amylase, Expert Opinion on Therapeutic Targets. 2012;16(3):269-297.
17. Farnsworth RN. Review on biological and phytochemical screening of plants, J Pharm Sci. 1966;55:225-276.
18. Gabbay KH. The Insulinopathies N Engl. J. Med. 1980;302:165-7.
19. Gerich JE. Matching Treatment to Pathophysiology in type 2 Diabetes, Clinical Therapeutics. 2001;23(5):646-659.
20. Grover JK, Yadav S and Vats V. Medicinal plants of India with anti-diabetic potential, Journal of Ethno pharmacology. 2002;81(1):81-100.
21. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis, 3rd ed, London, Chapman and Hall, 1998, 235.
22. Heinrich M, Barnes J, Gibbons S and Williamson EM, Fundamentals of Pharmacognosy and Phytotherapy. Churchill Livingston, Elsevier Science Ltd., U K. Herbal medicine and treatment of diabetes in Africa: an example from Guinea Diabetes Metabolism. 2005;32:171-175.
23. Hooper PL. Diabetes, Nitric oxide, and heat shock proteins Diabetes Care. 2003;26(3):951-952.
24. Houghton PJ, Raman A. Laboratory handbook for fractionation of natural extracts Chapman and Hall, London, 1998, 199.
25. Hussain A, Eshrat HM. Hypoglycaemic Hypoipidemic and antioxidant properties of combination of Cur cumin from Curcuma longa Linn and partially purified product from Abroma Augusta, Linn. In streptozotocin induced diabetes, Indian J. Clin. Biochem. 2002;17(2):33-43.
26. Hutchings A, Scott AH, Lewis G and Cunningham AB. Zulu Medicinal Plants, Natal University Press, Pietermaritzburg, 1996.
27. Iwu MM, Igboko OA, Okunji CO, Tempesta MS. Antidiabetic and aldose reductase activities of biflavanones of Garcinia Kola, Journal of Pharmacy and Pharmacology. 1990a;42:290–292.
28. Iwueke AV, Nwodo OFC. Ant hyperglycaemic effect of aqueous extract of Daniella Oliveri and Sarcocephalus latifolius roots on key carbohydrate metabolic enzymes and glycogen in experimental diabetes Biokemistri. 2008;20:63–70.
29. Joseph B, Raj SJ. Psychopharmacological properties of Ficus racemosa Linn - An overview, Int. J Pharm Sci. Rev Res. 2010a;3(2):134–138.
30. Josh CS, Priya ES, Venkataraman S. Acute and Sub acute studies on the polyhedral antidiabetic formulation Diakyur in experimental animal model, J. Health Sci. 2007;53(2):245-249
31. Kahu RC, White MF. The insulin receptor and the molecular mechanism of insulin action, J. Clin. Invest. 1988;82:1151.
32. Kaku K. Pathophysiology of type 2 diabetes and its treatment policy JMAJ. 2010;53(1):41-46.
33. Kim YM, Jeong YK, Wang MH, Lee WY, Rhee HI. Inhibitory effect of pine extract on α-Glucosidase activity and postprandial hyperglycaemia nutrition. 2005;21:756-761.
34. Kimura T, Suzuki J, Ichikawa M. Differential effects of α-Glucosidase inhibitors on postprandial plasma glucose

and lipid profile in patients with type 2 diabetes under control with Insulin Lispro Mix 50/50 Diabetes Technology Therapy. 2012;14:545-51.
35. Klassen CD, Amdur MO, Doull J. Casarett and Doull's Toxicology: The basic science of poison, 8th Edition, Mc Graw Hill, USA, 1995, 13-33.
36. Li W, Zheng H, Bukuru J, De Kimpe N. Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus, Journal of Ethno pharmacology. 2004;92(1):1-21.
37. Lin L, Park S, Lakatta EG. RAGE signalling in inflammation and arterial aging frontiers in bioscience. 2009;14:1403-1413
38. Lukic ML, Al-sharif R, Mostarica M, Bahr G, Behbehani K. Immunological basis of the strain differences in susceptibility to low-dose streptozotocininduced diabetes in rats. In: Lymphatic Tissues and In vivo Immune Responses. Eds. Imhof.; New York. Marcel Dekker Inc, 1991, 643-647.
39. Maiti R, Jana D, Das UK and Ghosh D. Antidiabetic effect of aqueous extract of seed of Tamarindus indicia in streptozotocin-induced diabetic rats, Journal of Ethno pharmacology. 2004;92(1):85-91.
40. Mbaze LM, Poumale HM, Wansi JD, Lado JA, Khan SN, Iqbal MC. A-Glucosidase inhibitory pent acyclic triterpenes from the stem bark of Fagara tessmannii (Rutaceae) Phytochem. 2007;68:591–5.
41. McCue PP, Shetty K. Inhibitory effects of rosmarinic acid extracts on porcine pancreatic amylase in vitro, Asia Pacific Journal of Clinical Nutrition. 2004;13(1):101-106.
42. McCue P, Kwon YI, Shetty K. Anti-diabetic and anti-hypertensive potential of sprouted and solid-state bioprocessed soybean, Asia Pacific Journal of Clinical Nutrition. 2005;14(2):145-152.
43. Mccue P, Kwon YI, and Shetty K. "Anti-amylase, anti glucosidase and anti-angiotensin I-converting enzyme potential of selected foods", Journal of Food Biochemistry. 2005;29(3):278–294.
44. McVeigh GE, Brennan GM, Johnston GD, McDermott BJ, McGrath LT, Henry WR *et al.* Dietary fish oil augments nitric oxide production or release in patients with type 2 (non-insulin-dependent) diabetes mellitus Diabetologia. 1993;36:33-38.
45. Mohammadi J, Naik PR. Evaluation of hypoglycaemic effect of Morus Alba in an animal model, Indian Journal of Pharmacology. 2008;40:15–18.
46. Mohammed RK, Ibrahim S, Atawodi SE, Eze ED, Suleiman JB. Anti- diabetic and haematological effects of n-butanol fraction of Alchornea cord folia leaf extract in streptozotocin-induced diabetic Wistar rats, Global Journal of Medicinal Plant Research. 2012b;1:14–21.
47. Molina JM, Cooper GIS, Leighton B, Olefsky JM. Induction of insulin resistance in vivo by amylin and calcitonin gene-related peptide Diabetes. 1990;39:260-5.
48. Muhammad S, Bierhaus A, Schwaninger M. Reactive Oxygen Species in Diabetes-induced Vascular Damage, Stroke, and Alzheimer's Disease, Journal of Alzheimer's Disease. 2009;16(4):775-785
49. Mythilypriya R, Shanthi P, Sachdanandam P. Oral acute and subacute toxicity studies with Kalpaamruthaa, a modified indigenous preparation, on rats, J. Health Sci. 2007;53(4):351-358.
50. Nagappa A, Thakurdesai P, Rao NV, Singh J. Antidiabetic activity of Terminalia catappa Linn fruits, Journal of Ethno pharmacology. 2003;88(1):45-50.
51. Ndukwe KC, Okeke IN & Lamikanra A. Antibacterial Activity of Aqueous Extracts of selected chewing sticks, J Contemp Dent Pract. 2005;6(3):086-09.
52. Nickavar B, Yousefian N. Inhibitory effects of six allium species on α- amylase enzyme activity, Iranian Journal of Pharmaceutical Research. 2009;8(1):53-57.
53. Ogbonnia Steve. Cardiovascular risk factors: Implications in Diabetes, Other Disease States and Herbal Drugs, The Cardiovascular System - Physiology, Diagnostics and Clinical Implications, Dr. David Gaze (Ed.), In Tech, 2012, DOI: 10.5772/30858.
54. Ogbonnia S, Adekunle AA, Bosa MK, Enwuru VN. Evaluation of acute and subacute toxicity of Alstonia congensis Engler (Apocynaceae) bark and Xylopia aethiopica (Dunal) A.Rich(Annonaceae) fruits mixtures used in the treatment of diabetes, African Journal of Biotechnology. 2008a;7:701–705.
55. Ogbonnia SO, Anyakora C. Chemistry and Biological Evaluation of Nigerian plants with Anti-Diabetic Properties, African natural plant products in New Discoveries and challenges in chemistry and quality, American Chemical Society, 2009, 185–207.
56. Ogbonnia SO, Adekunle A, Olagbende-Dada SO, Anyika EN, Enwuru VN, Orolepe M. Assessing plasma glucose and lipid levels, bodyweight and acute toxicity following oral administration of an aqueous Ethanolic extract of Parinari curatellifolia Planch, (Chrysobalanaceae) seeds in alloxan-induced diabetes in rats, African Journal of Biotechnology. 2008b;7:2998–3003.
57. Ogbonnia SO, Mbaka GO, Adekunle A, Anyika EN, Gbolade OE, Nwakakwa N. Effect of a poly-herbal formulation, Okudiabet, on alloxan-induced diabetic rats, Agriculture and Biology Journal of North America1., 2010, 139–145.
58. Ogbonnia SO, Odimegwu JI, Enwuru VN. Evaluation of hypoglycaemic and Hypoipidemic effects of aqueous Ethanolic extracts of Treculia Africana Decent and Bryophyllum pinnatum Lam. And their mixture on streptozotocin (STZ)-induced diabetic rats, African Journal of Biotechnology. 2008c;7:25-35.
59. Ojewole JAO. The hypoglycaemic effect of Clausea anisata (Wild) Hook Ethanolic root extracts in rats, Journal of Ethno pharmacology. 2002;81:231-237.
60. Ojewole JAO. Hypoglycaemic effect Sclerocarya birrea stem-bark aqueous extracts in rats Phytomedicine. 2003;10:675-681.
61. Ojewole JAO. Ant nociceptive, anti-inflammatory and antidiabetic effects of Bryophyllum pinnatum (Crassulaceae) leaf aqueous extract, Journal of Ethno-pharmacology. 2005;99:13–19.
62. Okonta JM, Aguwa CN. Evaluation of hypoglycaemic activity of glycosides and alkaloids extracts of Picralima Nitida Stapf (Apocynaceae) seed. International Journal of Pharmacology. 2007;3:505–509.
63. Olajide OA, Makinde JM Awe SO. Effect of aqueous extract of Bridelia ferruginea stem bark on carrageenan-induced oedema and granuloma tissue formation on rats and mice, J Ethnopharmacol. 1999;66(1):113-117.
64. Oputa R, Chinenye S. Diabetes mellitus: A global epidemic with potential solutions, African Journal of Diabetes Medicine, 20(2), 33. Medicinal Chemistry, 2012;24(6):1288-1290.
65. Oyaizu M. Studies on product of browning reaction: ant oxidative activities of products of browning reaction prepared from glucose amine JPN J Nutr 1986;44:307–31

66. Patel DK, Prasad SK, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property, Asian Pacific Journal of Tropical Biomedicine. 2012;2(4):320-330.
67. Patwardhan B, Vaidya DB and Chorghade M. "Ayurveda and natural products drug discovery", Current Science. 2004;86(6):789–799.
68. Puls W. Pharmacology of Glucosidase inhibitors. Oral Antidiabetics. Springer, Berlin. 1996;119:497–525.
69. Puls W, Keup U, Krause HP, Thomas G and Hofmeister F. Glucosidase inhibition: A new approach to the treatment of diabetes, obesity and hyperlipoproteinaemia. Naturwissenschaften. 1977;64:536-537.
70. Randle PJ, Garland PB, Hales CN and Newsholme EA. Lancet. 1963;1:785.
71. Sabitha V, Ramachandran S, Naveen KR, Panneerselvam K. Antidiabetic and antihyperlipidemic potential of Abelmoschus esculent us (L.) Moench.in streptozotocin-induced diabetic rats, Journal of Pharmacy and Bioallied Sciences. 2011;3:397–402.
72. Saidu AN, Mann A, Onuegbu CD. Phytochemical screening and hypoglycaemic effect of aqueous Blighia sapid a root bark extract on normoglycemic albino rats, British Journal of Pharmaceutical Research. 2012;2:89–97.
73. Sell DR, Nagaraj RH, Grandhee SK, Odetti P, Lapolla A, Fogarty J *et al*. Pentamidine - A Molecular marker for the cumulative damage to proteins in Diabetes, Aging, and Uraemia Diabetes - Metabolism Reviews. 1991;7(4):239-251.
74. Shimoda H, Kawamori S, Kawahara Y. Effects of an aqueous extract of Salacia reticulate, a useful plant in Sri Lanka, on postprandial hyperglycaemia in rats and humans, J Jpn Soc Nutr Food Sci. 1998;51:279-87.
75. Shobana S, Sreerama YN & Malleshi NG. Composition and enzyme inhibitory properties of finger millet (Eleusine Coracana L.) seed coat phenolics: Mode of inhibition of α-Glucosidase and pancreatic amylase Food Chemistry. 2009;115(4):1268-1273.
76. Smith KJ, Pagé V, Gariépy G, Béland M, Badawi G & Schmitz N. Self-rated diabetes control in a Canadian population with type 2 diabetes: Associations with health behaviours and outcomes, Diabetes Research and Clinical Practice. 2012;95(1):162-168.
77. Sofowora A. Screening plants for bioactive agents. In medicinal plants and traditional in Africa, Spectrum Books Ltd Ibadan, 2ed, 1993, 134-156.
78. Sushruta K, Satyanarayana S, Srinivas N and Sekhar RJ. Evaluation of the blood–glucose reducing effects of aqueous extracts of the selected Umbellifereous fruits used in culinary practice, Trop. J. Pharma. Res. 2006;5(2):613-617.
79. Soladoye MO, Chukwuma EC, Owa FP. An 'Avalanche' of plant species for the traditional cure of diabetes mellitus in South-Western Nigeria, Journal of Natural Products and Plant Resources. 2012;2:60–72.
80. Stratton IM, Neil HA, Yudkin JS. Association of systolic blood pressure with macro vascular and micro vascular complications of type 2 diabetes prospective observational study British Medical Journal. 2000;321:412-419.
81. Stratton IM, Adler AI, Neil HAW, Matthews DR, Manley SE, Cull CA et al. Association of glycaemia with macro vascular and micro vascular complications of type 2 diabetes (UKPDS 35): prospective observational study, British Medical Journal. 2000;321(7258):405-412.
82. Stryer JJ. Biochemistry Third Edition. Metabolism of carbohydrates Ch. 1990;12:346-377.
83. Teugwa CM, Mejiato PC, Zofou D, Tchinda BT, Boyom FF. Antioxidant and antidiabetic profiles of two African medicinal plants: Picralima nitida (Apocynaceae) and Sonchus oleraceus (Asteraceae), BMC Complementary and Alternative Medicine. 2013b;13:175.
84. Ugochukwu NH, Babady NE. Antihyperglycemic effect of aqueous and Ethanolic extracts of Gongronema latifolium leaves on glucose and glycogen metabolism in liver of normal and streptozotocin-induced diabetic rats Life Sciences. 2003;73:1925–1938.
85. Van Wyk BE, Van Oudtshoorn B and Gericke N. Medicinal plants of South Africa, 2nd Edition, Briza Publications, Pretoria, 1997, 246.
86. World Health Organization study group on diabetes mellitus. Technical report series No. 727. World Health Organization, Geneva, 1985, 1-108.
87. World Health Organization study group on diabetes mellitus. Technical report series No. 844, World Health Organization, Geneva, 1994;3:78-79.
88. Xu Heping, Curtis Timothy, Stitt Alan. Pathophysiology and pathogenesis of diabetic retinopathy Diapedia. 2013;7104343513:14
DOI:10.14496/dia.7104343513.1
89. Yakar S, Setser J, Zhao H, Stannard B, Haluzik M, Glatt V, LeRoith D. Inhibition of growth hormone action improves insulin sensitivity in liver IGF-1-deficient mice, Journal of Clinical Investigation. 2004;113(1):96-105.
90. Yates T, Khunti K, Wilmot EG, Brady E, Webb D, Srinivasan B, Davies MJ. Self-reported sitting time and markers of inflammation, insulin resistance, and adiposity, American Journal of Preventive Medicine. 2012;42(1):1-7.
91. Zavala AV, Fabiano de Bruno LE, Cardoso AI, Mota AH, Capucchio M, Poskus E *et al.* Cellular and humoural autoimmunity markers in Type 2 (non-insulin-dependent) diabetic patients with secondary drug failure Diabetologia. 1992;35:1159-11.
92. Quilliot D, Walter E & Bonte J. Diabetes mellitus worsens antioxidant status in patients with chronic pancreatitis, American Journal of Clinical Nutrition. 2005;81:1111–1117.
93. Nordestgaard BG, Zilversmit DB. Hyperglycaemias in normotriglyceridemic, hypercholesterolemia insulin-treated diabetic rats does not accelerate parthenogenesis Atherosclerosis. 1988;72(1):37-47.