IDENTIFICATION OF THE BIOACTIVE PHYTOCHEMICALS IN AQUEOUS AND ETHANOL LEAF AND ROOT EXTRACTS OF ALAFIA BARTERI PLANT

BY

OLAITAN PHILIP

17010102001

A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES, COLLEGE OF BASIC AND APPLIED SCIENCES, MOUNTAIN TOP UNIVERSITY, PRAYER CITY, OGUN STATE, NIGERIA

IN FUFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc.) DEGREE IN BIOCHEMISTRY

SEPTEMBER, 2021.

DECLARATION

I, Olaitan Philip Seun hereby declare that this project entitled **"Identification of the Bioactive Phytochemicals in Aqueous and Ethanol Leaf and Root Extracts of *Alafia Barteri* Plant"** is a record of my research work. Al sources of information have been specifically acknowledged

CERTIFICATION

It is hereby certified that this work was carried out by Olaitan Philip Seun, Matriculation Number 17010102001, of the Department of Biological Sciences, Biochemistry, Mountain Top University, Ogun State, Nigeria, under the supervision of Mrs.Ikeoluwapo Olanike Kolawole.

……..
……

Mrs. KOLAWOLE O.I Date

(Supervisor)

……..
……

Dr. O.T Kayode Date

Head of Department

ACKNOWLEDGEMENTS

Am forever grateful to God Almighty who has been my assistant and forte in this research project and the journey of badging an honorable degree.

My sincere appreciation goes to my project Supervisor Mrs.Ikeoluwapo Olanike Kolawole, who has worked round the clock to see that this research project work never became an abandoned project, imbibing in me the core values of being worthy in learning and in character. May God reward you graciously.

To the Head of the Department of Biological Sciences- Dr. O.T Kayode, thank you for being a mother and ensuring the laboratory equipment were supplied to the Departmental Laboratory.

To Mr. Abba Gabriel, the laboratory technologist in biochemistry laboratory, I really appreciate your great supports and rendering of assistance when called upon.

To Mr. O.O Ojo the chemistry laboratory technologist, I really appreciate your help and supports in all ramification.

My appreciation goes to other Biochemistry lecturers, Dr. F.J Femi-Olabisi, Dr. O.O Ayodele, Dr, T.O Kayode, Dr. E.E Okoro, Mrs. I.O Kolawole, Mr. Babalola Benjamin for their intellectual supports, may God bless you all.

I am thankful for the blessing of the family, whose moral, financial and courageous words show me daily love, kept me going and into becoming a better person. Mrs Olaitan, Ekere Daniella, and Opadeji Mary I love you all.

Also, I would like to appreciate my project colleagues, Amize Eben, Alabi Tolulope and Akintunde Amos. Who contributed enormously and took active part to make this project a work of success. I must say I really enjoy working with you all, I pray God help you all and help you actualize your dreams in time.

Ibeawuchi Innocent, Joshua Daniel, Adeyemi Tijesunnimi, Atoyebi Ayomide, Oladapo feranmi Olujimi folakemi, Banigo thammy and the whole of biochemistry department you all have proven to be more than friends, you all are my family, and I love you all

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

GC-MS- Gas Chromatography-Mass Spectrometry

AEABL- Aqueous Extract *Alafia bacteri* Leaves.

AEABR- Aqueous Extract *Alafia bacteri* roots.

EEABL- Ethanol extract of *Alafia bacteri* Leaves.

ABSTRACT

Alafia barteri is one of the medicinal plants of importance belonging to the family *Apocynaceae.* The aim of this study is to identify the bioactive phytochemical components in the different extracts of *A. barteri* leaves and roots using Gas chromatography-Mass spectrometry (GC-MS) analysis. Qualitative and quantitative determination of the different bioactive compounds from aqueous and ethanol leaf extracts of *A. barteri* revealed different types of high and low molecular weight chemical entities with varying quantities present in each of the extracts. The importance of identifying the most abundant bioactive phytochemicals is to induce into pharmacological activities for remediation of illness such as malaria, inflammation.

Key Words: *Alafia barteri*, GC-MS, Pharmacological

CHAPTER ONE

1.0 INTRODUCTION

Plants with therapeutic characteristics in their roots, stems, leaves, and seeds have been recognized as medicinal plants (Muhammad *et al.,* 2011). Many plants used in traditional medicine to treat sickness symptoms and have been discovered to contain phytochemicals (Burkill, 1985). Due to the fact that medicinal plants are known to contain some chemical compounds that can be utilized for therapy or to make medications, the usage of herbs and the hunt for drugs and dietary supplements derived from plants has intensified in recent years (Muhammad *et al.,* 2011). Medicinal plants have an important part in people's health; in fact, most modern pharmaceuticals are derived from them (Iwu, 1993).

The phytochemical components of a medicinal plant are more and more aware of their therapeutic potential (Turker and Usta, 2008). Active compounds from plants have been screened for new medicinal products and they have an efficient protection against various diseases especially chronic inflammatory diseases (Sheeja K and Kuttan G, 2007).*Alafia barteri* is grown in tropical and subtropical climates between West and Central Africa. The *barteri* specie is the most economically important and widely cultivated of the 23 species of the botanical family *Apocynaceae* and the genera *Alafia*, 15 of which occur in continental Africa and 8 in Madagascar (De Ruijiter, 2006).

Gas chromatography-mass spectrometry (GC-MS) is an analytical technique for separating mixtures of volatile organic compounds and identifying each constituent compound (Eleanora *et al.,* 2018). It combines two well-known analytical techniques, gas chromatography and mass spectrometry (Eleanora *et al.,* 2018). To separate complicated mixtures, gas chromatography employs a well-designed column with variable component retention time (Eleanora *et al*., 2018).

1.1 AIM AND OBJECTIVE OF THE STUDY

The aim of this study is to identify the phytocompounds present in *Alafia Barteri* in order to provide a scientific basis for its traditional use as well as to discover new biological and pharmacological activities of this plant.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Medicinal Plants

The term medicinal plants include various types of plants used in herbalism and plants that have some medicinal activities. Medicinal plants are the "backbone" of traditional medicine, as they are the source of our understanding of what is good and bad about modern medicine. Some of this medicinal plant include ginger and garlic

2.1.1 Ginger

Ginger belongs to *Zingiberaceae* family and is a flowering plant whose root is commonly used in medicine and as a spice. Ginger is widely cultivated all over many countries such as Nigeria, Taiwan, India, Jamaica and Bangladesh. Ginger is said to originate from the tropical rainforests of the Indian subcontinent. It is reported to grow best in warm climates (Schauenberg P *et al.*, 1977).

2.1.2 The medical importance of ginger

Reports shows ginger has several medical importance in which they are:

Anti-ulcer and anticholinergic**,** Antioxidant, anti-inflammatory and rheumatologic properties; Analgesic effect; Blood circulation and anti-cramp effects; Lipid regulation and hypertensive properties; Antimicrobial effects. (Chinedu. I and Jivini Z *et al.*, 2019)

2.1.3 Garlic

Garlic (Allium sativum) is among the oldest of all cultivated medicinal plants.For many years, it has been used as a spice, food, and folklore. Garlic is native to Central Asia and north-eastern Iran and has long been a common seasoning worldwide. (Block Eric *et al.*, 2010)

2.1.4 The medical importance of garlic

Report has shown that garlic as several medical importance such as Treatment of cardiovascular disease; Reduction of Atherosclerosis and hyperlipidaemia; Immunity booster; Anti-diabetic property: Regulation of blood pressure; Antimicrobial effects; (. Duarte MC *et al*., 2016)

Aside from their high nutritional value, they have been reported to have antioxidant, anti-inflammatory, rheumatologic, blood circulation booster, and anti-cramp properties (Block Eric *et*

al., 2010). The use of these medicinal plant materials will contribute to the advancement of the human health system.

2.2 Alafia barteri

Alafia barteri is a species of *Alafia Olive*, a climbing shrub of the *Apocynaceae* family that can be found throughout the tropics. *A. barteri (Apocynaceae)* is a small, pure white or pink flowering shrub with a glabrous, scandent habit and a high climbing habit (Irvine, 1961). It is found throughout the tropics and is native to West and Central Africa, ranging from Guinea Bissau to Cameroon, Congo, and Nigeria (Irvine, 1961)

The natives of South-Western Nigeria (Lagos) call *A. barteri* agbari-etu, which translates as "instant fever remedy." It is highly valued in Nigerian and other African traditional medicine systems for its effectiveness as an anti-inflammatory and fever remedy. An infusion of the leaves and twining stem is used to treat inflammation and fever (Burkill, 1985; Iwu, 1993).

The *Apocynaceae* family includes the *Alafia barteri* plant. This plant is also known as Guinea fowl's crest, agbari-etu, otanza, ota, momunimo, ndambi, Alafia chewing stick, and other names in different parts of the world. *Apocynaceae* is a large family with over 200 genera and 2000 species, including *Alafia,* Catharanthus, Alstonia, and others (Irvine, 1961). Plants in the *Apocynaceae* family are poisonous, high in alkaloids, glycerides, and flavonoids derived from the leaves, seeds, stems, roots, and latex, and have anti-malarial properties (Leeuwenberg, 1997; Siu Kuin *et al*., 2011). It is indigenous to West and Central Africa, ranging from Guinea Bissau to Cameroon, Congo, and Nigeria.

A. barteri is grown in tropical and subtropical climates between West and Central Africa. The *barteri* specie is the most economically important and widely cultivated of the 23 species of the botanical family *Apocynaceae* and the genera *Alafia,* 15 of which occur in continental Africa and 8 in Madagascar (De Ruijiter, 2006). It is found in lowland forest from sea level to 200 metres (700 feet) in elevation (De Ruijiter, A., 2006).

2.2.1 Taxonomy of *Alafia barteri*

Kingdom: Plantae

Phyllum: Tracheophyta

Class: Magnolopsida

Order: Gentianales

Family: Apocynaceae

Genus: *Alafia*

Specie: *barteri*

Scientific name: *Alafia barteri* Oliv.

Source: GBIF Backbone Taxonomy (2021).

The Common name for *A. barteri* (*Apocynaceae)* is known as agbari-etu (immediate fever treatment), loko or mende (Sierra Leone), anyi (Ivory Coast), akan-asante or Fante (Ghana), otanza (Igbo) (Leeuwenberg, 1997). It is regarded in Nigerian and other African countries' traditional medicine systems for its usefulness as an anti-inflammatory and cure fever (Sofidiya *et al.,* 2014 and Lasisi *et al.,* 2016).The leaf extracts was discovered to have antibacterial and antifungal properties (Hamid and Aiyelaagbe, 2011).

Due to its efficacy in African traditional medicine, it is now used to treat sickle cell anemia, eye infections, toothaches and several disorders. (Odugbemi, 2008).The plant's leaf extracts were discovered to have antibacterial and antifungal properties, as well as anti-plasmodial and anti-diabetic properties. Its root and stem extracts have also been demonstrated to have anti-proliferative and analgesic properties (Hamid *et al* 2017; Ishola *et al.,* 2014). Research has shown that it has an impact on spermatogenesis and steroidogenesis (Adelakun *et al.,* 2018)

A. barteri is a species of *Alafia Olive,* a mounting shrub of the Apocynaceae family that can be found throughout the tropics. It is regarded in Nigerian and other African countries' traditional medicine systems for its usefulness as an anti-inflammatory and cure fever (Sofidiya *et al.,* 2014 and Lasisi *et al.,* 2016)). Inflammation and fever can be treated with an infusion of the leaves and winding stem (Hamid and Aiyelaagbe, 2011). The leaf extracts was discovered to have antifungal, antioxidant and antibacterial properties (Hamid and Aiyelaagbe, 2011). The anti-plasmodial activity of the aqueous root and leaf extract has been reported that *A. barteri* is a plant that has been used to cure malaria in South-Western Lagos (Nigeria) (Olowokudejo, 2018). Furthermore, *A. barteri* stem and root decoctions are used to cure rheumatic symptoms, toothaches, eye infections, and sickle-cell anemia (Olowokudejo, 2018).

There is a growing evidence of a correlation between a medicinal plant's phytochemical constituents and its pharmacological activity (Turker and Usta.2008).Screening of active compounds from plants has led to the invention of new medicinal drugs and they have an efficient protection against various diseases especially chronic inflammatory diseases (Sheeja K and Kuttan G, 2007).

2.3 Gas Chromatography-Mass Spectrometry Analysis

Gas chromatography-mass spectrometry (GC-MS) is an analytical technique for separating mixtures of volatile organic compounds and identifying each constituent compound (Eleanora *et al.,* 2018). It combines two well-known analytical techniques, gas chromatography and mass spectrometry (Eleanora *et al*., 2018). To separate complicated mixtures, gas chromatography employs a well-designed column with variable component retention time (Eleanora *et al*., 2018). The isolated chemicals can then be assessed using various detectors connected to the GC column's end (Eleanora *et al*., 2018).A mass spectrometer is used as the detector in GC-MS, allowing each compound form to be identified (Eleanora *et al*., 2018).

2.3.1 Principle of GC-MS

The GC-MS technique was developed by Robert Finnigan in the 1960s and is used to study liquid, gaseous, and solid samples (Igwe *et al*., 2016). A capillary column coated with a stationary (liquid or solid) phase is used in a gas chromatograph (GC) to efficiently vaporize the sample into the mobile phase and separate it into its various components (Igwe et al., 2016). The compounds are propelled by an inert carrier gas such as helium, hydrogen, or nitrogen. As the mixture's components are separated, each compound elutes from the column at a different rate depending on its boiling point and polarity. The time it takes for a chemical to elute is its retention time (Igwe *et al*., 2016).Because it combines a rapid separation approach with a time-dependent identification module, the GC-MS offers a broader method for characterization of organic compounds present in plant extracts MS (Igwe *et al*., 2016).

2.3.2 Application of GCMS

GC-MS has an extensive range of applications in a diversity of scientific fields (Grant, 2021). In environmental science, GC-MS is often used to assess the amounts of organic pollutants (Grant, 2021). It's been used to discriminate between different types of hydrocarbons in a sample for bioremediation and testing (Grant, 2021).In forensic toxicology and criminal cases, as well as drug detection in law enforcement, GC-MS has been used (Grant, 2021). The approach has been used in explosives research, anti-doping and drug detection, and astrochemistry for the study and evaluation of samples collected from distant moons in our solar system (Grant., 2021).GC-advantages MS's include improved sample detection, sensitivity, a wider range of analyzable

samples, and faster performance, allowing for a wide range of new applications in a variety of industries (Susha, 2019). Medicine, environmental monitoring, food and fragrance analysis, pharmaceutical use, forensic application, biological analysis, chemical warfare, geological study, and industrial use are only a few of the possible applications for this technology (Susha, 2019)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Apparatus and Reagents

Beakers, Warring blender, Oven, Rotatory Evaporator, measuring cylinder, Spatula, Sieve, beakers, gloves, Ethanol

3. 2 Collection and authentication of the plant material

The complete plant material of *Alafia Barteri* was collected in Osun State in January 2021 identified at the Botany department of the University of Lagos by Dr. Nodza George where a voucher specimen number: LUH 8789 was allotted to it.

3.3 Preparation of plant extracts

Aqueous and ethanol extractions of *A. barteri* root bark were performed at Mountain Top University's Biochemistry Laboratory in Ogun State. The leaves and root barks were washed, air dried; blended into powdery form and chopped into small pieces respectively.60 grams of both the chopped root bark and powdered leaves were weighed into two glass jars each containing 300mls of distilled water and 300mls of ethanol. The mixtures were macerated at room temperature for 72 hours with occasional vigorous stirring. Filtration was carried out using a muslin cloth and the different filtrates were collected in clean beakers.

The aqueous filtrate was concentrated in the laboratory oven at 45^{o}C while the ethanol filtrate was concentrated with a rotary evaporator.

3.4 Gas Chromatography-Mass Spectrometry Analysis

GC-MS analysis was carried out on a GC system comprising a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) instrument; Schimadzu GCMS-QP2010, employing the following conditions: Column Elite-1 fused silica capillary column (30×0.25 mm ID×1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) as carrier gas at a constant flow of 1ml/minute and a sample injection volume of 1µl which was employed (split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed to start at 110°C (isothermal for 2 minutes), then rise at a rate of 10°C/minute to 200°C, then 5°C/minute to 280°C, with a 9-minute isothermal at 280°C. Mass spectra were collected at 70 eV with a scan interval of 0.5s and fragments ranging in size from 40 to 550 Da. The total duration of the run was 30 minutes. The compounds were then identified from the GC-MS peaks using library data from the corresponding compounds.

GC-MS was analyzed using electron impact ionization at 70 eV, and data was analyzed using total ion count (TIC) for compound identification and quantification. NISP Search was used to compare the component spectrums to a database of known component spectrums stored in the GC-MS library. The relative % amount of each component was calculated by comparing its average peak area to the total areas. Measurement of peak areas and data processing were carried out by Turbo-Mass-OCPTVS-Demo SPL software

CHAPTER FOUR

4.0 RESULTS

Percentage Yield: $\frac{\text{Weight after extraction}}{\text{Weight before extraction}} \times 100$

Aqueous extract yielded 83.3 %w/w with a brown colour while the ethanol extract yielded 66.6% with dark green colour.

4.1 BIOACTIVE COMPONENTS PRESENT IN EXTRACTS OF *ALAFIA BARTERI*

The bioactive compounds present in the aqueous and ethanol leaf and root extracts of AB are shown in table 1, table 2 and table 3.Their identification were based on their elution order in the column. The elution time and the amount of these bioactive compounds were also presented. Based on abundance, the top 4 major compounds present in the aqueous leaf extract were stigmastan-6,22-dien,3,5-dedihydro(28.5%),n-Hexadecanoicacid(15.9%),Ergost-5-en-3-ol,(3.beta.)(13.41.%)and9,12,octadecadienoic acid(11.47%).The ethanol leaf extract contained 3 major compound based on abundances of phytochemical they are 2-pyrrolidinone,1 methyl(12.03%),cyclotetrasiloxane,octamethyl(12.56%)and 9,12-octadecadienoic acid(8.12%) while the aqueous root extract have 2 major compounds high in phytochemical constituent which is n-Hexadecanoic acid(35.93%) and 6-octadecenoic acid(24.77%)

The GC chromatograms of the extracts presented in figure1, figure2 and figure 3 show he retention time in the column and the detected peaks which correspond to the bioactive compounds present in the extract. The interpretation of the mass spectrum was done by National

Institute Standard and Technology Database (NIST) database that contained more than 62,000 patterns. The X axis represents the retention time of each compound identified in minutes while the Y-axis represents the intensity or the presence of various compounds with a corresponding percentage of peaks area at different retention times.

4.2 GC-MS ANALYSIS OF AQUEOUS EXTRACT OF *ALAFIA BARTERI* LEAVES

Table 1. GC-MS result of aqueous extract of *Alafia barteri* leaves

Peak No	Retention Time	Area	%Compound
1	3.398	1.19	Benzene,1-ethyl-3-methyl-
2	3.554	0.40	5-methyl-6-phenyltetrahydro-1,3-oxazine-2-thione
3	3.776	0.41	2(Acetyl) oxybenzylidene acetophenone.
4	3.876	0.32	1,3,8-p-menthrieneBenzene, 1,4-dimethyl-
5	4.154	0.26	1-Methyl-1-silabenzocyclobutene
6	4.242	0.28	Benzene, 1-ethyl-2,3-dimethyl-
7	4.442	1.43	Undecane
8	4.709	1.01	Benzene,1,2,3,5-tetramethyl-
9	5.053	0.23	Benzene,1,3-diethyl-5methyl-
10	5.142	0.26	6,7 –Dimethyl-3,5,8a-tetrahydro-1 H-2-benzopyran
11	5.209	0.24	4-Dehydroxy-N-(4,5-methylenedioxy nitrobenzylidene) tyramine
12	5.276	0.16	2H-1,3-benzoxazine-6-carboxylic acid, 3,4-dihydro-3-methyl-, methyl ester
13	5.331	0.42	2H-1,3-benzoxazine-6-carboxylic acid, 3,4-dihydro-3-methyl-, methyl ester
14	5.476	0.15	2-Amino-6-methoxy-4-(2H-1,2,3 tetrazol-5-yl)phenol
15	5.720	0.35	Hexahydropyridine, 1-methyl-4-[4,5-dihydroxyphenyl]-
16	5.831	0.82	Dodecane, Undecane, Octadecane
17	6.020	0.21	Piperazine,1,2,4-trimethyl-9-Borabicyclo[3.3.1]nonane,
18	6.320	0.19	2H-1,3-benzoxazine-6-carboxylic acid 3,4-dihydro-3-methyl-, methyl ester
19	7.198	0.18	1-Methoxy-3-hydroxymethylheptane Dodecahydropyrido [1,2-b]isoquinolin-6 one
20	8.420	0.20	Cyclooctaneacetic9-
21	9.664	0.42	Borabicyclo[3.3.1]nonane,9-[3

			(dimethylamino)propyl]-acid,
22	9.953	0.44	2,4-Di-tert-butylphenol Phenol, 2,6-bis(1,1-dimethylethyl)
23	10.864	0.15	Undec-10-ynoic acid, dodecyl ester
24	11.319	0.30	Benzene,(1-butylheptyl)-5,6-Azulenedicarboxaldehyde,
25	12.064	0.27	2-(n-Pentyl)oxybenzylidene acetophenone
26	12.264	0.32	Trichloroacetic acid, undec-2-enyl ester
27	12.375	0.20	cis-4-Ethoxy-b-methyl-b-nitrostyrene2-(Methylpropyl)oxybenzylideneacetophenone [1,2,4] Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 7-amino-, ethyl ester
28	12.408	0.17	2-(2-Methylpropyl)oxybenzylideneacetophenone Benzene,(1-ethyldecyl)-2-(n-Pentyl)oxybenzylidene acetophenone
29	12.864	0.99	Tetradecanoic acid
30	13.052	0.15	N-(2-Acetylcyclopentylidene)cyclohexylamine 4H-1,2,4-triazole-3,5-diamine,N3-(4 fluorophenyl)-N5-methyl2-Piperidinone, N-[4-bromo-n-butyl]-
31	13.164	0.33	Benzoic acid, 2-(methylamino)-,2- methylpropyl ester 2-Myristynoyl-glycinamide Acetic acid, oxo((1-phenylethyl)amino)-, hydrazide
32	13.741	0.23	1,1,3,3-Tetraallyl-1,3-disilacyclobutane 2 Myristynoyl-glycinamide Benzazirene-1-carboxylic acid, 2,2,5a-1-trimethyl-1a-[3-oxo-1-butenyl] perhydro-, methyl ester
33	13.886	0.69	2H-3,9a-Methano-1-benzoxepin,octa hydro 2,2,5a,9-tetramethyl-, [3R (3.alpha,5a.alpha,9a.alpha.,9a.alpha.)]-2H-1,3-benzoxazine-6-carboxylic acid, 3,4-dihydro-3 ,methylester 3,5Dimethylbenzaldehydethiocarbamoylhydrazone.

34	14.330	0.15	[1,2,4]Triazolo[1,5-a]pyrimidine-6 -carboxylic acid, 4,7-dihydro-7-imino- ,ethylester3,5Dimethylbenzaldehydethiocarbamoylhydrazone; 2-Myristynoyl-glycinamide
35	14.652	0.49	2-Myristynoyl-glycinamide1,2,5-Oxadiazol-3-amine,4-(3-methoxyphenoxy)-2-Methyl-Z,Z-3,13-octadecadienol
36	14.863	15.96	n-Hexadecanoic acid
37	15.074	0.62	Cyclohexaneethanol,4-methyl-.beta .-methylene-, transUndec-10-ynoic acid, undecylester 2-Methyl-Z,Z-3,13octadecadienol
38	15.152	0.75	13-Octadecenal, -3,5 Dimethylbenzaldehydethiocarbamoylhydrazone 11-Tetradecyn-1-ol acetate
39	15.330	0.29	dimethyl-3-(prop-1-en-2 yl)octahydronaphthalen-1(2H)-one1,2,5-Oxadiazol-3-amine4-(3-methoxyphenoxy) 3,5Dimethylbenzaldehydethiocarbamoylhydrazone
40	15.397	0.16	1-Benzazirene-1-carboxylicacid,2,2,5a-trimethyl-1a-[3-oxo-1-butenyl]perhydro-, methylester2-Myristynoyl-glycinamide 1,1,3,3-Tetraallyl-1,3-disilacyclobutane
41	15.463	0.28	2-Myristynoyl-glycinamide1H-Imidazole, ethyl-1,2,5-Oxadiazol-3-amine,4-(3-methoxyphenoxy)-
42	15.641	0.60	[1,2,4]Triazo[1,5-a]pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester 1,2,5-Oxadiazol-3-amine, 4-(3-methoxyphenoxy) -3,5-Dimethylbenzaldehydethiocarbamoylhydrazone

43	15.708	0.21	2-Myristynoyl-glycinamide 1,2,5 -Oxadiazol-3-amine, 4-(3-methoxyphenoxy) -3,5-Dimethylbenzaldehydethiocarbamoylhydrazone
44	15.808	0.68	(5S,6aR,10aS)-5-Propyldecahydrodipyrrolo [1,2-a:1',2'-c]pyrimidine Isolongifolan-8-ol 4-Allyl-5-furan-2-yl-2,4-dihydro-[1,2,4]triazole-3-thione
45	15.908	0.56	2-Methyl-Z,Z-3,13-octadecadienol Cyclopropaneoctanoicacid,2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester 9-Methyl-Z,Z-10,12-hexadecadien-1-ol acetate
46	16.052	0.25	2-Myristynoyl-glycinamide[1,2,4]Triazolo[1,5 a] pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester 2-Penta-2,4-dienyl-cyclohexanecarboxylic acid, methyl ester
47	16.185	0.26	1,2,5-Oxadiazol-3-amine, 4-(3-methoxyphenoxy) 9-Methyl-Z,Z-10,12-hexadecadien-1-ol acetate 5,9-Dimethyl-2-(1-methylethylidene)-1-cyclodecanol
48	16.263	0.19	1,2,5-Oxadiazol-3-amine,4-(3-methoxyphenoxy)- Cyclopropaneoctanoicacid,2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, Methylester3,5-Dimethylbenzaldehydethiocarbamoylhydrazone.
49	16.463	11.47	9,12-Octadecadienoic acid
50	16.730	4.38	7-Pentadecyne 8-Hexadecenal, 14-methyl-,Bromoacetic acid, Tetradecylester
51	17.074	0.16	Cyclopropaneoctanoic acid,

52	17.152	0.34	2-Methyl-Z,Z-3,13-octadecadienol 7-Hexadecyn-1-ol 9,12-Octadecadien-1-ol,
53	17.230	0.20	2-Myristynoyl-glycinamide1,2,5-Oxadiazol-3-amine, 4-(3-methoxyphenoxy)- 4,7,7-Trimethylbicyclo[2.2.1]heptan-2-one O-allyl oxime
54	17.430	0.27	2-Myristynoyl-glycinamide1,2,5-Oxadiazol-3-amine,4-(4-methoxyphenoxy)-Cyclopropaneoctanoicacid,2-[[2-[(2ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester
55	17.718	0.15	2-Myristynoyl-glycinamides1-methyl-4-phenyl-5-thioxo-1,2,4-t riazolidin-3-one
56	18.641	0.20	2-Myristynoylglycinamide[1,2,4]Triazolo[1,5-a]pyrimidine-6 -carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester Cyclotrisiloxane,hexamethyl-
57	18.852	0.29	(R)-(-)-14-Methyl-8-hexadecyn-1-ol2-Methyl-Z,Z-3,13-octadecadienol Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester
58	19.663	0.31	3,5-Dimethylbenzaldehydethiocarbamoylhydrazone 4,7,7-Trimethylbicyclo[2.2.1]n-2-one O-allyl oxime
59	19.874	13.41	Ergost-5-en-3-ol, (3.beta.)-9-(2,3-Epoxypropoxy)-1,2,3,4-tetrahydroacridineSilane, dimethyl(3-fluorophenoxy) tetradecyloxy
60	20.18	1.86	Cholesta-6,22, 24-triene, 4,4-dimet -hyl2-Methyl-4-(2,6,6-trimethylcyclohex-1-enyl)but-2-en-1-ol Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)-

61	20.429	0.45	3,5Dimethylbenzaldehydethiocarbamoylhydrazone 1-Benzazirene-1-carboxylicacid,,2,5a-trimethyl-1a-[3-oxo-1-butenyl]perhydro-methylester1,2,5-Oxadiazol-3-amine, 4-(3-methoxyphenoxy)
62	20.629	0.24	3,5Dimethylbenzaldehydethiocarbamoylhydrazone Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl4,7,7-Trimethylbicyclo[2.2.1]hepta
63	20.896	28.05	Stigmastan-6,22-dien,3,5-dedihydro Stigmasterol Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)
64	21.140	1.77	3,5-Dimethylbenzaldehydethiocarbamoylhydrazone 2-Pyridinamine,N-(4,5-ethyl-2-thiazolyl)-3-methyl1,1,3,3-Tetraallyl-1,3-disilacyclobutane
65	21.618	1.16	Cyclotrisiloxane, hexamethyl- Acetamide, N-[4-(trimethylsilyl)phenyl]- 4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine
66	21.740	0.24	3-Methylindole-2-carboxylicacid,4,5,6,7-tetrahydro-, ethyl ester 7-Methyl -2-phenyl-1H-indole 1H-Indole, 6-methyl-2-phenyl-
67	21.818	0.16	2-Myristynoyl-glycinamide2-Pyridinamine, N-(4,5-dihydro-5-m ethyl-2-thiazolyl)-3-methyl-[1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester

4.3 GC-MS CHROMATOGRAMS OF AQUEOUS EXTRACT OF *ALAFIA BARTERI* LEAVES

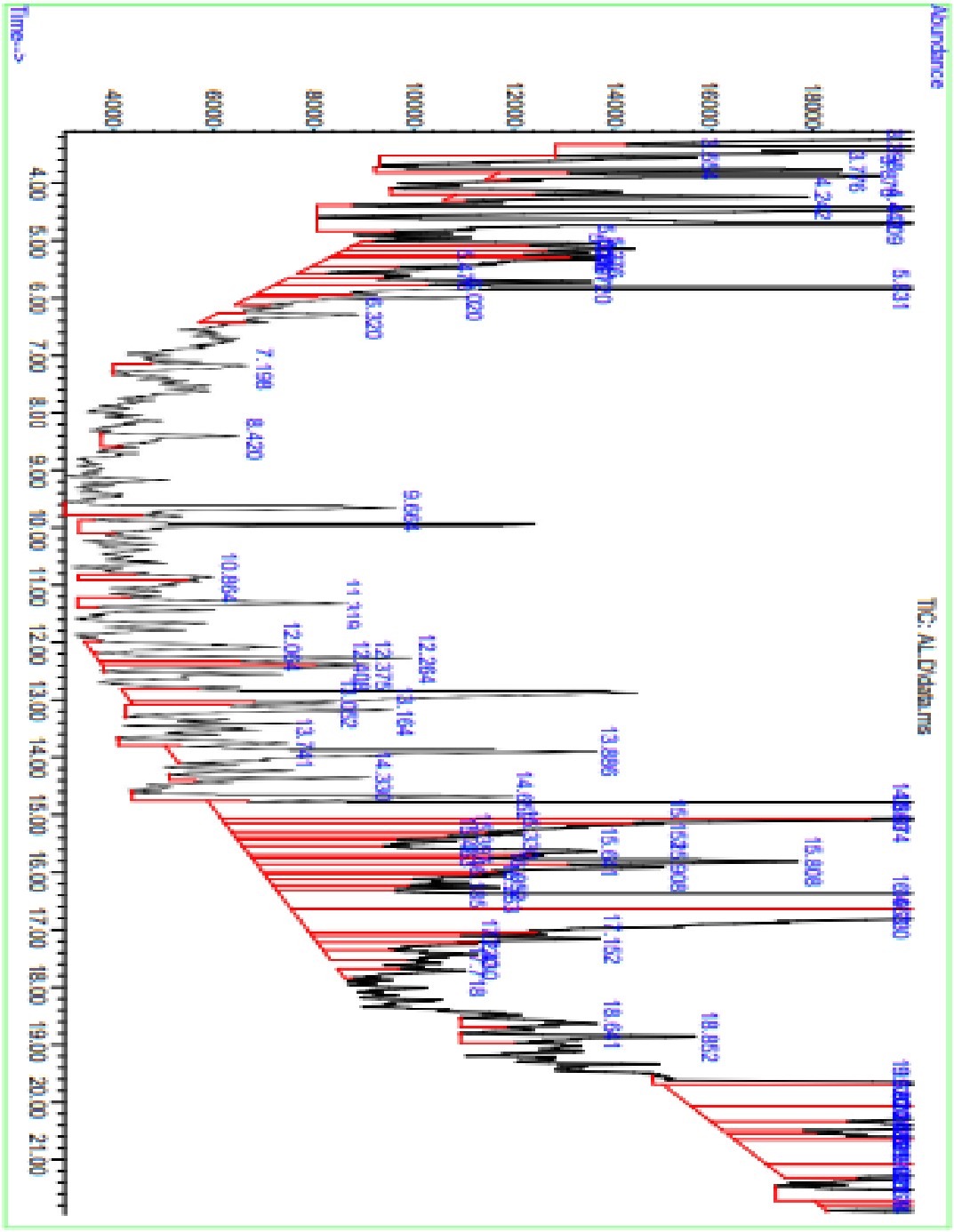

FIGURE 1: Chromatogram result of aqueous extract of *Alafia barteri* leaves

4.4 GC-MS ANALYSIS OF ETHANOL EXTRACT OF *ALAFIA BARTERI* LEAVES

Table 2: GC-MS results of ethanol extract of *Alafia barteri* leaves

Peak No	Retention Time	Area %	Compound
1	3.276	12.56	Cyclotetrasiloxane, octamethyl- 2,6-Dihydroxyacetophenone, 2TMS derivative Cyclotetrasiloxane, octamethylde
2	3.565	0.37	2-Fluoroethyl acrylate Ethanol, 2-(methoxyethylthio)- 2-Undecanone
3	3.642	0.99	Formic acid, 2,4-dimethylpent-3-yl ester 2-Pentyn-1-ol Formic acid, 1-methylpropyl ester
4	4.99	4.99	1-Hexene, 1-chloro-, (E)- Dimethallyl ether 2-Aminocyclohexanol
5	3.965	3.80	Hydroxylamine, O-(2-methylpropyl)- 1-Propanol, 3-mercapto- Cyclohexanol, 2-amino-, cis-
6	4.231	0.42	Oxirane, (3-methylbutyl)- 2-Undecanone, 6,10-dimethyl- 3-Methoxy-4,5-methylenedioxy- N-methylamphetamine
7	4.465	0.15	p-Cresol 1,2-Cyclooctadiene Phenol, 3-methyl-
8	4.531	0.21	Mequinol2-Cyclopenten-1-one, 2,3,4-trimethyl Phenol, 2-methoxy-
9	4.920	14.03	2-Pyrrolidinone, 1-methyl-
10	5.187	0.92	2-Pyrrolidinone, 1-(3,7,11-trimethyldodecyl)- Piperazine, 1-[2-(2,5-dimethyl-1H-pyrrol-1-yl)ethyl]-
11	5.342	1.27	Cyclopentasiloxane, decamethyl-
12	5.587	0.65	Propanenitrile, 3-(5-diethylamino-1-methyl-3-pentynyloxy)- 2,3,4,6-Tetrafluorophenyl isothiocyanate 4-Bromo-2,6-difluoroaniline
13	6.020	0.31	Hexanoic acid, hexyl ester 3-Heptene
14	6.075	0.20	.alpha.-Pyrrolidinopropiophenone

			Benzoic acid
15	6.553	0.17	3-Chloro-1,1,2,2-tetrafluoropropan 2-Propylthiazole Hexanoic acid, 2-methylpropyleste
16	6.742	0.17	1-Tri(isobutyl)silyloxytridecane Silane, Diethyl (trans-4-methylcyclohexyloxy)undecyloxy 1H-Pyrazole-1-acetamide, 4-iodo-N- (4-pyridinylmethyl)-
17	6.875	0.33	9-Borabicyclo[3.3.1]nonane, 9-(1-ethylpropyl)- 3-Buten-2-one, 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)- 2,5-Dichloro-4-methoxy-pyridin-3-ylamine
18	7.264	0.47	Succinic acid, hept-2-yl oct-1-en 3-yl ester
19	7.486	0.95	Cyclotetrasiloxane, octamethyl- trans-4-(2-(5-Nitro-2-furyl)vinyl) -2-quinolinamine 1,1,3,3,5,5,7,7-Octamethyl-7-(2-me thylpropoxy)tetrasiloxan-1-ol
20	7.698	0.53	Acetoxyacetic acid, 5-tetradecyl ester 3-Nitrophthalhydrazide Succinic acid, docosyl isobutyl ester
21	7.897	0.31	N-Methyl-1-adamantaneacetamide 2-quinoxalinamine, 3-chloro-N-ethyl- 1,2,5-Oxadiazole-3-carbonitrile, 4-amino-
22	9.497	0.75	2-Ethyl-3-methoxypyrazine 2,5-Dihydroxybenzaldehyde 3-Methoxy-5-methylphenol
23	9.697	0.57	2,3-Methylenedioxyphenol 1,2,4,5-Benzenetetramine 2,5-Cyclohexadiene-1,4-dione, dioxime
24	9.819	0.44	2,3-Dihydroxybenzaldehyde 19-Norethindrone, O-methyloxime 2,5-Dihydroxybenzaldehyde
25	9.931	0.32	4-Fluoro-2-(trifluoromethyl)benzamide Terephthalic acid, 2-methoxyethyl propyl ester Carbendazim
26	10.053	0.34	2(5H)-Furanone, 4,5,5-trimethyl-3-(3-methyl-2-methylenebutyl)- dimethyl-mercapto-arsine 2,5-Dihydroxybenzaldehyde
27	10.131	0.27	5-(2-Aminoethoxy)-2H-1,3-benzodioxole Cyclohexa-2,5-diene-1,4-dione, 2-methyl-5-(4-morpholinyl)- 3H-Benzofuran-2-one, 3,7-dihydroxy, 3,7-dihydroxy-3-trifluoromethyl

28	10.730	0.28	1-Benzazirene-1-carboxylic acid, 2 ,2,5a-trimethyl-1a-[3-oxo-1-buteny l] perhydro-, methyl ester 1H-Indole, 5-methyl-2-phenyl-
29	11.419	2.27	Benzeneacetic acid, 2,5-dihydroxy- 5,5 Dimethyl-3-vinyl cyclohex-2-en-1-one 4(5H)-Benzofuranone, 6,7-dihydro-3,6-dimethyl-, (R)-
30	11.475	3.29	Benzeneacetic acid, 2,5-dihydroxy- Formic acid phenyl ester 4(5H)-Benzofuranone, 6,7-dihydro-3,6-dimethyl-, (R)-
31	11.786	2.50	Benzeneacetic acid, 2,5-dihydroxy- 2-Methyl-6,7-dihydro-5H-benzofuran-4-one Benzo[b]thiophene-2-ol
32	11.897	0.52	Benzeneacetic acid, 2,5-dihydroxy-Oxirane, (phenoxymethyl)- 2-Methyl-6,7-dihydro-5H-benzofuran-4-one
33	11.975	0.51	Benzeneacetic acid, 2,5-dihydroxy- Oxirane, (phenoxymethyl)- 5,6,7,8-Tetrahydro-1,2,4-benzotriazine-3-amine
34	12.053	0.70	Benzeneacetic acid, 2,5-dihydroxy- 5,6,7,8-Tetrahydro-1,2,4-benzotriazine-3-amine Benzo[b]thiophene-2-ol
35	12.230	0.71	Benzeneacetic acid, 2,5-dihydroxy- 2,5-Methano-1H-inden-7(4H)-one, hexahydro- Propanoic acid, phenyl ester
36	12.319	0.66	Benzeneacetic acid, 2,5-dihydroxy- Tricyclo[4.2.2.0(1,5)]decan-4-one Borolo[1,2-a]borine, octahydro-
37	13.286	0.21	2(3H)-Naphthalenone, 4,4a,5,6,7,8-hexahydro 9-Borabicyclo[3.3.1]nonane, 9-[3-(dimethylamino)propyl]- 4,5,6,6a-Tetrahydro-2(1H)-pentalenone
38	13.486	0.23	Cyclohexa-2,5-diene-1,4-dione, 2-methyl-5-(4-morpholinyl)- Benzoic acid, 2,4-dihydroxy-6-methyl-, methyl ester Oxirane, (phenoxymethyl)-
39	13.663	0.32	Hex-5-ynoic acid, methyl ester 1-Octen-3-yne Oxirane, (phenoxymethyl)-
40	13.841	0.60	Pentadecanoic acid Ethyl n-butyl disulphide Bicyclo[3.2.2]nona-2,6-dien-5-ol-4-one
41	14.663	0.78	2-Myristynoyl-glycinamide

			3-pyridinamine, 2-[(4-methyl-4H-1, 2,4-triazol-3-yl)thio]- Ethyl n-butyl disulphide
42	14.852	7.97	n-Hexadecanoic acid
43	15.019	0.30	Pyrido[2,3-d]pyrimidine, 4-phenyl- [1,2,4]Triazolo[1,5-a]pyrimidine, 2-ethylsulfanyl-5,7-dimethyl Dodecahydropyrido[1,2-b]isoquinolin-6-one
44	15.319	0.20	Bicyclo[6.1.0]non-1-ene [1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester Bicyclo[5.2.0]non-1-ene
45	15.408	0.15	Tricyclo[4.2.1.1(2,5)]decane 1-Cyclopentenylphenylmethane Pentacyclo[6.3.0.0(2,7).0(4,11).0(5,9)]undecan-3-one
46	15.674	1.94	Stigmasta-4,22-diene 2-Myristynoyl-glycinamide 1,2,4-Oxadiazole, 3-(1,3-benzodioxol-5-yl)-5-[(4-iodo-1H-pyrazol-1-y l)methyl]-
47	15.796	1.46	Stigmasta-4,22-diene Ergosta-4,7,22-trien-3.beta.-ol Stigmasta-5,22-dien-3-ol, acetate, 2(3.beta.)-
48	15.908	0.98	7-Chlorobicyclo[4.1.0]hept-3-ene 4,7-Methano-1H-indene, octahydro- Stigmasta-4,22-diene
49	15.974	1.21	Stigmasta-4,22-diene 1,2,4-Oxadiazole, 3-(1,3-benzodioxol-5-yl)-5-[(4-iodo-1H-pyrazol-1-y l)methyl]- Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)-
50	16.096	1.10	Ergost-4,7,22-trien-3.alpha.-ol Stigmasta-3,5-diene 1,2,4-Oxadiazole, 3-(1,3-benzodioxol-5-yl)-5-[(4-iodo-1H-pyrazol-1-y l)methyl]-
51	16.096	0.35	4-Allyl-5-furan-2-yl-2,4-dihydro-[1,2,4]triazole-3-thione 1,2,5-Oxadiazol-3-amine, 4-(3-methoxyphenoxy)- 4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine
52	16.463	8.12	9,12-Octadecadienoic acid (Z,Z)- 9,12-Octadecadienoic acid (Z,Z)- 9,17-Octadecadienal, (Z)-
53	16.719	1.19	2(1H)-Naphthalenone, octahydro-4a-methyl-7-(1-methylethyl)-, (4a. alpha, 7.beta,8a.beta.)- 2-Methyl-Z,Z-3,13-octadecadienol

			4,7,7-Trimethylbicyclo[2.2.1]heptan-2-one O-allyl oxime
54	18.207	0.21	1,2,5-Oxadiazol-3-amine, 4-(3-methoxyphenoxy)- 3,5Dimethylbenzaldehydethiocarbamoylhydrazone [1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester
55	18.274	0.18	3,5- Dimethylbenzaldehydethiocarbamoylhydrazone Isolongifolan-8-ol 1,2,5-Oxadiazol-3-amine, 4-(3-ethoxyphenoxy)-
56	18.429	0.86	1,2,5-Oxadiazol-3-amine, 4-(3-methoxyphenoxy)- [1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester 3,5- Dimethylbenzaldehydethiocarbamoylhydrazone
57	18.652	1.18	1,2,5-Oxadiazol-3-amine, 4-(3-methoxyphenoxy)- 3,5- Dimethylbenzaldehydethiocarbamoylhydrazone Ethanone, 2-(2-benzothiazolylthio) -1-(3,5-dimethylpyrazolyl)-
58	19.918	0.34	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)- 3-methyl-4-oxo-4,5,6 ,7-tetrahydro-, isopropyl ester Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy- 3,3-dimethyl 2-(n-Propyl)oxybenzylidene acetophenone
59	19.074	0.37	3,5-Dimethylbenzaldehyde thiocarbamoylhydrazone Silicic acid, diethyl bis(trimethylsilyl) ester 4-Allyl-5-furan-2-yl-2,4-dihydro-[1,2,4]triazole-3-thione
60	19.585	0.15	1,2,5-Oxadiazol-3-amine, 4-(3-methoxyphenoxy)- 3,5- Dimethylbenzaldehydethiocarbamoylhydrazone 2-Myristynoyl-glycinamide
61	19.585	0.49	3,5-Dimethylbenzaldehyde 1thiocarbamoylhydrazone 2-(n-Propyl)oxybenzylidene acetophenone [1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester
62	19.825	1.34	2-Methyl-4-(2,6,6-trimethylcyclohex-1-enyl)but-2-en-1-ol 2-Methyl-6-(5-methyl-2-thiazolin-2-ylamino)pyridine Benzoic acid, 4-(1,3-dioxolan-2-yl)-, methyl ester
63	20.029	0.37	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-

			3-methyl-4-oxo-4,5,6 ,7-tetrahydro-, isopropyl ester Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl N-Methyl-1-adamantaneacetamide
64	20.229	0.88	3-Isopropyl-6a,7,10b-trimethyl-dodecahydro-benzo[f]chromene-7,8-dicarboxylic acid, dimethyl ester 1,2,5-Oxadiazol-3-amine, 4-(3-methoxyphenoxy)- 4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine
65	20.374	0.45	1,2,5-Oxadiazol-3-amine, 4-(3-methoxyphenoxy)- 2-Butenenitrile, 2-chloro-3-(4-methoxyphenyl)- Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl
66	20.563	1.22	1,2,5-Oxadiazol-3-amine, 4-(3-methoxyphenoxy)- [1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester 2-(n-Propyl)oxybenzylidene acetophenone
67	20.829	3.23	Stigmastan-6,22-dien, 3,5-dedihydro-Stigmasterylosylate Cholesta-6,22,24-triene, 4,4-dimethyl-
68	21.107	0.70	1-Benzazirene-1-carboxylic acid, 2,2,5a-trimethyl-1a-[3-oxo-1-buteny l] perhydro-, methyl ester Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl 1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6 ,7-tetrahydro-, isopropyl ester
69	21.562	2.39	Cholesta-3,5-diene
70	21.807	0.60	Pyridine-4-carboxylic acid, 1,2-dihydro-3-cyano-5,6-dimethyl-2-oxo-, methyl ester benzeneacetaldehyde, .alpha.-(methoxymethylene)-4-nitro Furan-2-carboxylic acid, [4-(4-methoxyphenyl)-tetrahydropyran-4-ylmethyl]amide

4.5 GC-MS CHROMATOGRAM OF ETHANOL EXTRACT OF *ALAFIA BARTERI* LEAVES

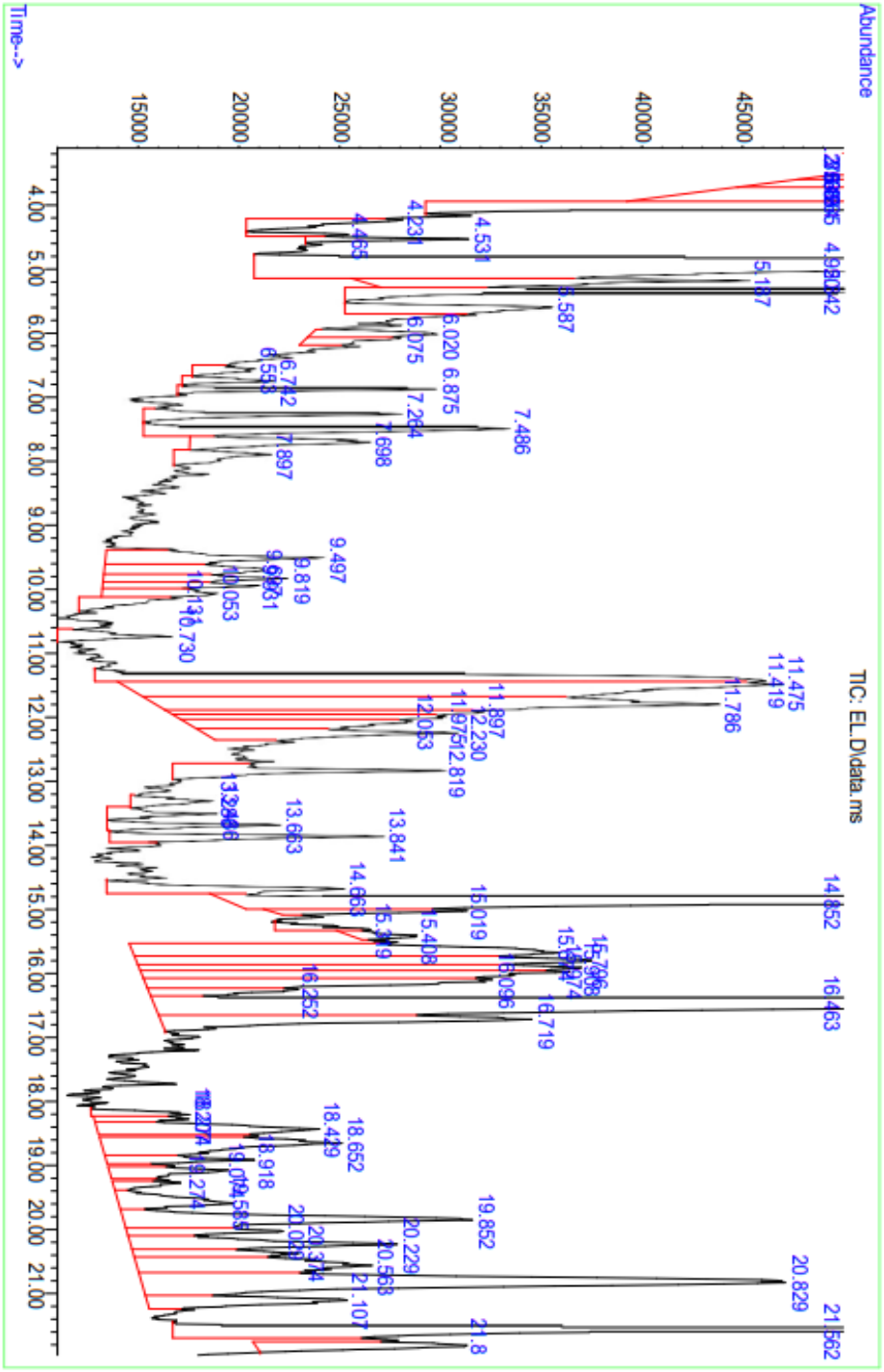

Figure 2: Chromatogram result of ethanol extract of leaf of *Alafia barteri* leaves

4.6 GC-MS ANALYSIS OF AQUEOUS EXTRACT OF *ALAFIA BARTERI* ROOTS

Table 3 GC-MS analysis result of aqueous extract of *Alafia barteri* roots

Peak No	Retention Time	Area%	Compound
1	3.049	0.84	Benzene, 1-ethyl-3-methyl-
2	3.476	0.35	Methyl10,12-pentacosadiynoate (4R,S)-4-(2-Butyl)-cis-bicyclo[4.3.0]-2-nonen-8-oneIsophthalic acid, 2-methoxyethylisobutyl ester
3	3.776	0.32	10-Undecen-1-al, 2-methyl-1,2,5-Oxadiazol-3-amine,4-(3-methoxyphenoxy)- Cyclooctene,1,2-dimethyl
4	4.253	0.48	1,3-Cyclopentadiene, 1,2,3,4-tetra methyl-5-methyleneBenzene, 1,2,3,5-tetramethyl- 1,3,8-p-Menthatriene
5	4.453	1.22	Undecane
6	4.731	0.83	1,3-Cyclopentadiene,1,2,3,4-tetramethyl 5methyleneBenzene,1,2,4,5-tetramethyl-3-Methyl2,3dihydrobenzofuran
7	5.842	0.44	Dodecane Decane Pentadecane, 2,6,10-trimethyl-
8	9.697	0.58	Spiro[2.5]octane, 3,3-dimethyl-2-(1-buten-3-on-1-yl)-2-Phenylethylsilane Pyrazolo[1,5-a]pyridine, 3,3a,4,7-tetrahydro-3,3-dimethyl-, (3aS)-
9	9.975	0.37	Phenol,3,5-bis(1,1-dimethylethyl)
10	10.886	0.57	Heptadecanolide.trans-2-Dodecen-1-ol,trifluoroacetate Cyclopentadecanone, 2-hydroxy-

11	12.097	0.77	9,12-Octadecadienoicacid (Z,Z)-,phenylmethylester Tricyclo[3.3.1.1(3,7)]decanone, 4-(acetyloxy)-, (1.alpha.,3.beta.,4. alpha.,5.alpha.,7.beta.)-1,2,5-Oxadiazol-3-amine,4-(3-methoxyphenoxy)9
12	12.263	0.70	Dichloroacetic acid, undec-2-enyl ester Octadecanal
13	12..375	0.35	Benzene, (1-pentylheptyl)-
14	12.541	0.40	2-Myristynoyl-glycinamide 2-Hydroxychalcone [1,2,4]Triazolo[1,5-a]pyrimidine-6 -carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester
15	12.863	2.24	Tetradecanoic acid
16	13.163	0.48	1,2,5-Oxadiazol-3-amine,4-(3-metoxyphenoxy)- 2-Myristynoyl-glycinamide N-(2Acetylcyclopentylidene)cyclohexylamine
17	13.508	1.01	Undec-10-ynoicacid, dodecylesterOleyl alcohol, trifluoroacetateZ)-Tetradec-11-en-1-yl 2,2,3,3,3- pentafluoropropanoate
18	13.585	0.54	Undec-10-ynoic acid, dodecyl ester 9-Octadecenoic acid8-Hexadecenal, 14-methyl-,
19	13.73	0.47	2-Methyl-Z,Z-3,13-octadecadi enol (S)(+)-Z-13-Methyl-11-pentadecen-1-ol acetate 11,13-Dimethyl-12-tetradecen-1-ol acetate
20	13.885	2.30	Pentadecanoic acid
21	14.508	0.33	cis-Vaccenic acid cis-13-Octadecenoic acid 2-Methyl-Z,Z-3,13-octadecadienol

22	14.674	1.45	(1S,15S)-Bicyclo[13.1.0]hexadecan-2-one 7-Pentadecyne 8-Hexadecenal, 14-methyl-, (Z)-
23	14.919	35.93	n-Hexadecanoic acid
24	15.641	0.78	Bicyclo[3.3.2]decan-9-one cis-7,cis-11-Hexadecadien-1-ylacetate Ethanol, 2-(9-octadecenyloxy)-, (Z)-
25	15.819	0.94	Undec-10-ynoic acid, undecyl ester cis-11,12-Epoxytetradecen-1-ol Heptadecanoic acid
26	15.930	0.85	Cyclopropaneoctanoic acid, 2-[[2-[53 (2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methylester 2-Methyl-Z,Z-3,13-octadecadienol Cyclohexene, 1-nonyl-
27	16.496	24.77	6-Octadecenoic acid cis-Vaccenic acid 9-Octadecenoic acid
28	16.752	9.59	Decanoic acid, 10-(2-hexylcyclopropyl) 1-Pentadecene 9Cyclopentadecane
29	16.985	1.63	9,12-Octadecadienoic acid (Z,Z)- Linoelaidic acid 9,12-Octadecadienoic acid (Z,Z
30	17.385	0.40	Z,Z-4,15-Octadecadien-1-ol acetate 7-Pentadecyne 9,12-Octadecadienoic acid (Z,Z)
31	17.485	0.55	2-Methyl-Z,Z-3,13-octadecadienol 13-Octadecenal, (Z)- cis-11-Hexadecenal
32	18.863	0.32	4,7,7-Trimethylbicyclo[2.2.1]heptan-2-one O-allyloxime Bromoacetic acid, octadecylester

			Bromoacetic acid, hexadecylester
33	19.574	0.33	2-Myristynoyl-glycinamide [1,2,4]Triazolo[1,5-a]pyrimidine-6 -carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester Indole-2-one, 2,3-dihydro-N-hydrox y-4-methoxy-3,3-dimethyl
34	19.662	0.32	Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl2-Myristynoyl-glycinamide [1,2,4]Triazolo[1,5-a]pyrimidine-6 -carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester
35	19.807	0.55	Diglycolic acid, di(2-isopropylphenyl) ester 2-Myristynoyl-glycinamide 5H-dibenzo[a,d]cyclohepten-5-amine
36	20.251	0.90	1,2,5-Oxadiazol-3-amine, 4-(3-methoxyphenoxy)- [1,2,4]Triazolo[1,5-a]pyrimidine-6 -carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester 2-Pyridinamine, N-(4,5-dihydro-5-methyl-2-thiazolyl)-3-methyl
37	20.729	0.68	4H-1,2,4-triazole-3,5-diamine, N3-(4-fluorophenyl)-N5-methyl-[1,2,4]Triazolo[1,5-a]pyrimidine-6 -carboxylic acid, 4,7-dihydro-7-imino-,ethylester1,1,1,3,5,5,5-Heptamethyltrisiloxane
38	20.885	20.885	1-Nitro-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid diethyl amide 2-Myristynoyl-glycinamide4-Dehydroxy-N-(4,5-methylenedioxy2-nitrobenzylidene)tyramine
39	20.973	0.42	benzeneacetaldehyde,.alpha.-(methoxymethylene)-4-nitro3H-indole,

			2-methyl-3-phenyl 1H-Indole, 1-methyl-2-phenyl-
40	21.162	1.44	Isolongifolan-8-ol Methyl(5-hydroxy-1H-benzimidazol2-yl)carbamate 1H-Indole, 1-methyl-2-phenyl
41	21.262	0.29	2-Myristynoyl-glycinamide [1,2,4]Triazolo[1,5-a]pyrimidine-6 -carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester 4,7,7-Trimethylbicyclo[2.2.1]hepta 7n-2- O-allyloxime

4.7 GC-MS CHROMATOGRAM OF AQUEOUS EXTRACT OF *ALAFIA BARTERI* ROOT

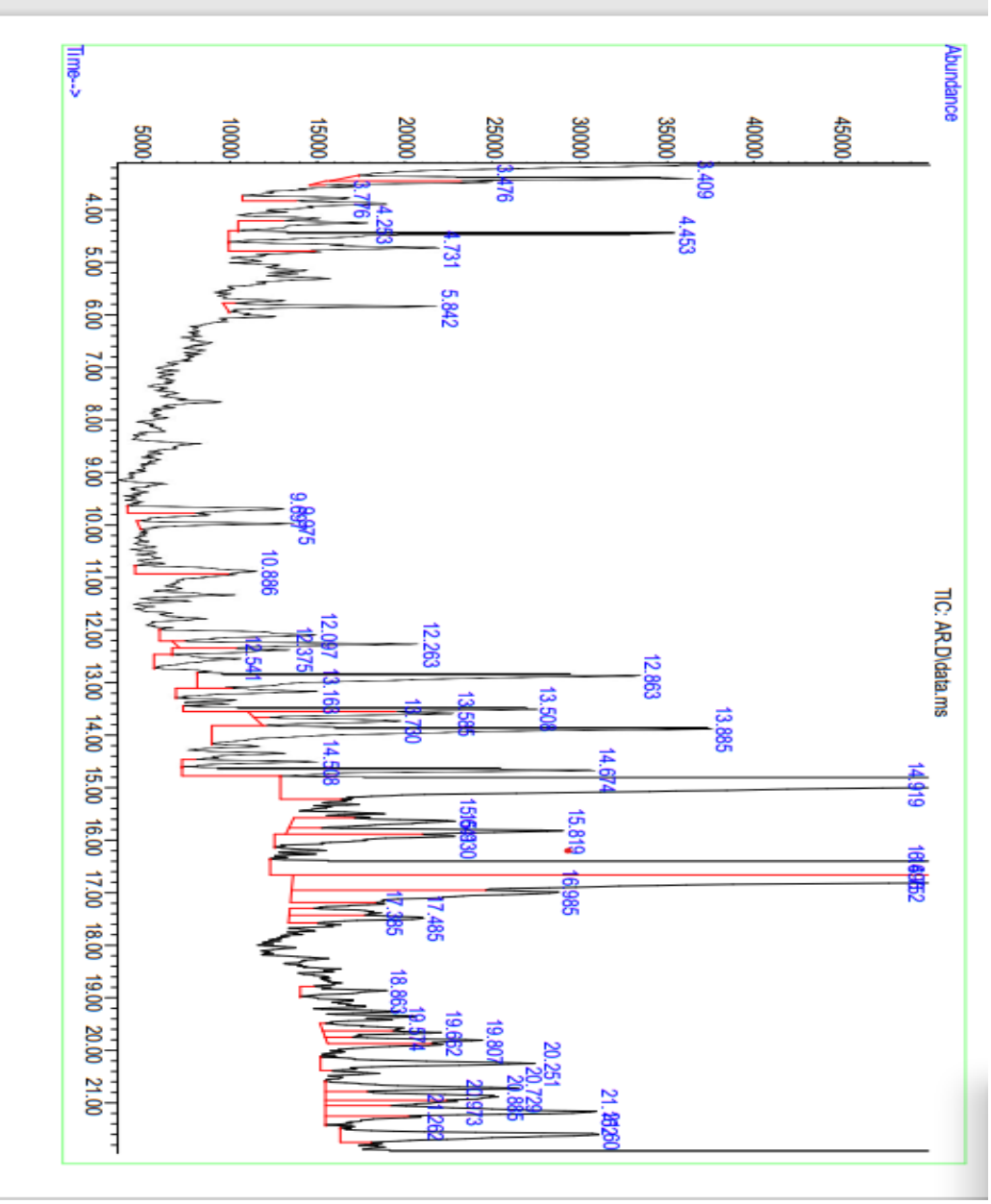

Figure 3. Chromatogram of aqueous extract of *Alafia barteri* roots

4.7 MAJOR BIOACTIVE COMPONENTS IN *ALAFIA BARTERI* AND THEIR REPORTED BIOACTIVITY

Table 4. Result of major bioactive compounds in *Alafia barteri*

Name of Compound (AL)	**Area %**	**Molecular Formula**	**Molecular Weight(g/mol)**	**Reported Bioactivity**
Ergost-5-en-3-ol,(3beta)	13.41	$C_{28}H_{48}O$	400.694	Anti-cancer, Anti-oxidant (Choudhary D, *et al* 2019) Jaundice, Arthrosclerosis
n-Hexadecanoic acid	15.96	$CH_3(CH_2)_{14}CO_2CH$	270.45	Antioxidant(Reuben et al., 2021) Antifungal,(Chandrasekaran *et al*.,2021) Anti-inflammatory(Aparna *et al.*, 2012)
Stigmasten-6,22,-dien,3,5-dedihydro-	28.05	$C_{29}H_{48}O$	412.705	Synthetic Progesterone, anti-oxidant(Berger A *et al* 2003)
9,12-octadecadienoic acid	11.47	$^{13}C_5C_{13}H_{32}O_2$	285.41	Anti-microbial and anti-oxidant activity(Nuerxiati *et al.*,2021), and anti-cancer(uy *et al.*, 2005)

Name of Compound (EL)	**Area %**	**Molecular Formula**	**Molecular Weight(g/mol)**	**Reported Bioactivity**
Cyclotetrasiloxane,octamethyl	12.46	$[-Si(CH_3)_2O-]_4$	296.62	Anti-cancer (Lutfia *et al.*,2021; Babalola, *et al*., 2021), Anti-fungal (Lotfi *et al.*, 2021) anti-bacteria (Hussin *et al*.,2021)
2-pyrrolidinone,1 methyl	14.03	C_5H_9NO	99.13	No information.

9,12-octadecadienoic acid	8.12	$^{13}C_5C_{13}H_{32}O_2$	285.41	Anti-microbial and anti-oxidant activity(Nuerxiati *et al.*,2021), and anti-cancer(uy *et al.*, 2005)
n-Hexadecanoic acid	7.97	$CH_3(CH_2)_{14}CO_2CH_3$	270.45	Antioxidant(Reuben et al., 2021) Antifungal,(Chandrasekaran *et al*.,2021) Anti-inflammatory(Aparna *et al.*, 2012)
1-hexene,1-chorol	4.99	$CH_3(CH_2)_3CH=CH_2$	84.16	Anti-cancer(Reuben et al., 2021) anti-diarrhea

Name of Compound (AR)	**Area %**	**Molecular Formula**	**Molecular Weight(g/mol)**	**Reported Bioactivity**
n-Hexadecanoic acid	35.93	$CH_3(CH_2)_{14}CO_2CH_3$	270.45	Antioxidant(Reuben *et al*.,2021) Antifungal,(Chandrasekaran *et al*.,2021)
6-octadecenoic acid	24.77	$CH_3(CH_2)_{10}CH=CH(^{13}CH_2)_4{}^{13}COOH$	287.42	Antiandrogenic, anti-cancer, (Lutfi *et al*.,2021) anti-inflammatory (s.)
9,12-octadecadienoic acid	1.63%	$^{13}C_5C_{13}H_{32}O_2$	285.41	Anti-microbial and anti-oxidant activity(Nuerxiati *et al*.,2021)
Pentadecanoic acid	2.30	$CH_3(CH_2)_{13}COOH$	242.40	Lubricants, Adhesive agents(Chandrasekeran et al., 2021)
Tetradecanoic acid	2.24	$CD_3(CH_2)_{12}CO_2H$	231.39	Antioxidant, (Alkhamis *et al* 2021)Anticancer(Dockerill *et a*l.,2021) Hypocholesterolemi

CHAPTER FIVE

5.0 DISCUSSION

The combination of gas chromatography with mass spectrometry (GC-MS) provides more precise information for qualitative analysis (Cong Z *et al*, 2007). Several phytochemical screening studies using GC-MS analysis have been conducted in various parts of the world. In identifying the bioactive compounds in plants, the GC-MS is considered a proven technique as it suggests the plant's possible curative properties (Uraku, 2015). In compound detection, the GC-MS is used to separate, qualify and identify the analytes present in the plant sample (Maurer, 1995)

In this study, it was observed that the phytochemical compounds present in *Alafia barteri* varies in abundance, and consequently, bioactivity. The result on the aqueous leaf extract of *Alafia barteri* showed that there are 67 phytochemical compounds in the extract, of which the abundant phytochemical compounds are stigmastan-6,22-dien,3,5-dedihydro-(28.05%), n-Hexadecanoic acid (15.96%), Ergost-5-en-3-ol(13.41%) and 9,12-octadecadienoic acid(11.47%).

The bioactive compounds in the aqueous leaf of *A.barteri* have reported bioactivity as anti-oxidant, anti-inflammation (Choudhary D, *et al* 2019*)*, antidiabetic, antidiarrheal and anti-cancers (Reuben *et al*., 2021). 70 phytochemical compounds were found in the ethanol leaf extract, of which only six are most abundant compounds, having a significantly high peak values, when analyzed from the chromatogram of the GC-MS result. These compounds are cyclotetrasiloxane, octamethyl(12.56%), 2-pyrrolidinone, 1methyl(14.03%) ,9,12-octadecanoic acid(8.12%), n-Hexadecanoeic acid(7.97%), Benzenoacetic acid, 2,5-dihydroxy-(3.29%) and 1-hexene,1-chorol(4.99).The bioactive compounds in ethanol leaf of *A.barteri* have anti-oxidant, anti-microbial, anti-cancer and anti-diarrhea((Lutfia *et al.*,2021; Babalola, 2021), properties used in treatment of various illnesses.

The aqueous root extract of *Alafia barteri* contained majorly n-Hexadecanoic acid(35.93%), 6-octadecanoic acid(24.77%),9,12-octadecadienoic acid(1.63%), pentadecanoic acid(2.30%)and tetradecanoic acid(2.24%)which have been reported to have anti-histaminic, antieczemic , hypocholesterolemic (Nuerxiati *et al*.,2021) and anti-anticancer properties.

5.1 CONCLUSION

The important pharmacological activities of *A. barteri* leaf and root extracts may be attributed to the presence of these bioactive components.

REFERENCES

Adeeyo OA, Caxton-Martins EA, Ofusori DA, Ashamu EA, Omotoso EO et al. (2008) Comparative histological features of the pancreas in fruit – eating bat (Eidolon – helvum) and Pangolin (Manis Tricuspis). J Cell and Animal Bio 2: 134 – 139. [View Article]

Adefisan, I.O., Ebuehi, A.O.T., Odesanmi, O.S., (2020). "Proximate, Mineral Composition and Phytochemical Screening of Aqueous Leaf Extract of Alafia barteri Oliv. (Apocynaceae)". 29(9): 108-112, Article no. IJBCRR.62896

Adekunle AA, Okoli SO (2002) Antifungal activity of the crude extracts of Alafia barteri. AGRIS. [View Article]

Adeyeye EI, Okokit MKO. Proximate composition and some nutritional valuable mineral of two varieties of Capsicum annum (Bell and Cherrypeppers). Discovery Innovation.1999. 11:75-81.

Antia BS, Akpan EJ, Okon PA, Umoren UI. Proximate composition and phytochemical constituents of leaves of some Acalypha species. Park J Nutr. 5:166-168

Babalola, B. A. (2020). Role of entrepreneurship in molecular oncology for sustainable development of the world's market. MTU Journal of Entrepreneurship and Sustainable Development.2 (1): 87-96.

Burkill HM., (1985). "The useful plants of West Tropical Africa. (Families A-D) ". Royal Botanic Gardens, Kew. 1:353-354.

Dalziel JM (1937).The useful plants of West Tropical Africa. Crown Agents for Overseas Governments and Administrations, London, United Kingdom. pp: 612.

Dockerill, M., Gregson, C. and Donovan, D.H. (2021). Targeting PRC2 for the treatment of cancer an update patent review (2016-2020). Expert Opinion on Therapeutic Patents, 31(2):119-135

Ebong PE, AtanguhoI J, Eyong EU, Egbung GE (2008) The antidiabteic efficiency of combined extracts from two. Continental plants: Azadirachta indica (A. Juss) (Neem) and Vernonia amygdalina (Doc) (African. Bitter leaf). AmJ Biochem Bitoechnol 4: 239-244.

Leuwenberg AJM (1997) Series of revisions of Apocynaceae XLIII. Alafia Thouars Kew Bulletin 52: 769–839.

Ebuehi OAT, Anams C, Gbenle DO, Ajagun-Ogunleye OM. Hydro-ethanol seed extract of Theobroma cacao exhibits antioxidant activities and potential anti-cancer property. J Food Biochemistry. 2019;43(4):1-10. Available: http//doi.org./10.1111/jfbc.1276.

Ebuehi OAT, Oyewole AC. Effect of cooking and soaking on physical characteristics, nutrient composition and sensory evaluation of indigenous and foreign rice varieties in Nigeria. Nutrition and Food Science. 2008;38 (1):15-21.

Eiziril DL Sandler S, Ahnstrom G and Wesh M (1991) Exposure of pancreatic islet to different alkylating agents decreases mitochondrial. DNA content but only streptozotocin inducers long-lasting functional impairment of B-cells. Biochem Pharmacol 42: 2275-2282.

Hamid AA., Aiyelaagbe OO., (2011). "Preliminary phytochemical, antibacterial and antifungal properties of alafia barteri stem grown in Nigeria". European Journal of Medicinal Plants. 1(2):26-32.

Hassan LG, Umar KJ. Nutritional value of balsam apple (Moordica balsamina L.) leaves. Pak J Nutr. 2006.5:522-529.

Irvine FR (1961) Woody plants of Ghana, with special reference to their uses. Oxford University Press, London, United Kingdom. pp: 868. [View Article]

Ishida H, Suzuno H, Sugiyama N, Innami S, Todokoro T. (2000). "National evaluation of chemical component of leaves stalks and stem of sweet potatoes". (Ipomea batatas Poir). Food Chemistry. 68:359–367.

Kim J, Knag S, Park G (2007) Ameliorative ant diabetic activity of dangnyosolao, Chinese herbal Medicine in diabetic rat. Bio Sci Biotechnol Biochem 71: 1527-1534.

Kimble SM, Joystick GS, Kamala PL, Vida SM (1996) effecacy of Coccinia indica WandA in Diabetes mellitus. J Res Ayurveda Sridhar 17: 77-84.

Lasisi AA., Olayiwola MA., Balogun SA., Akinloye OA., Ojo DA., (2016). "Phytochemical composition, cytotoxicity and in vitro antiplasmodial activity of fractions from Alafia barteri olive (Hook f Icon) Apocynaceae". J Saudi Chem Soc. 20(1):2-6.

Muhammad A, Dangoggo SM, Tsafe AI, Itodo AU, Atiku FA. Proximate, minerals and anti-nutritional factors of Gardenia aqualla (Guadendutse) fruit pulp. Pakistan Journal of Nutrition. 2011;10(6): 577-581.

Nuerxiati, R., Wubulikasimu, A., Mukhamedov,N.et al.,(2021.). Biological Activity of Fatty Acids from Lipids of *Orchischusua. Chem Nat Compd.* 57:230-230.

Ogbe AO, John P Affiku. Proximate Study, mineral and anti-nutrient composition of Moringa oleifera leaves harvested from lafia, Nigeria. Potential benefits in Poultry nutrition and health. Journal of Microbiology, Biotechnology and Food Sciences. 2011; 1(3):296-308.

Okwu DE, Okwu ME. Chemical Composition of Spondias mombin plants. J Sustain Agric. Environ. 2004; 6:140-147.

Olowokudejo JD, Kadiri AB, Travih VA. An ethnobotanical survey of herbal markets and medicinal plants in Lagos State of Nigeria. Ethnobotanical Leaflets. 2008;12:851-865.

Ozturk Y, Atlan VM, Vildizoglu N (1996) Effect of experimental Diabetes and insulin on smooth muscle functions. Pharmacol Rev 48: 69-112.

Rao CV, Newmark, HL. (1998). "Chemo-preventive effect of squalene on colon cancer". Carcinogenesis. 19:287-290.

Sofidiya MO., Essien I., Aigbe FR., (2014). "Antinociceptive and anti-inflammatory activities of ethanolic extract of Alafia Barteri Baker". Brazillian Journal of Pharmacognosy. 24: 348-354.

Sofowora A. Medicinal plant and traditional medicine in Africa. Ibadan-Owerri-KadunaLagos. Spectrum Book Ltd. 1993;158.

Yamagishi S, Maeda S, Matsui T, Ueda S, Fukami K, et al. (2012) Role of advance glycation end production (AGEs) and oxidative stress in vascular complication in diabetes. Biochimica of Biophysica Acta 1820: 663-671.

Yusuf Uthman A, Olusola A Adeeyo, Emmanuel O Salawu, Bernard U Enaibe, Olusegun D. Omotoso (2012) Allium cepa Protects Renal function in Diabetic Rabbit. World J Life Sci and Med Res 2: 86-90.